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Frank Robert Smith. 1856–1938 431
Electrocardiographic Changes in Acute Hemorrhagic Nephritis. Russell D. Williams, M.D. 434
Cyanosis in Chickens and Mice Induced by Sulfanilamide. Arthur P. Richardson 445
The Coincidence of Diabetes Mellitus and Addison's Disease. Effect of Cortical Extract on Glycemia and Glycosuria. A. L. Bloomfield, M.D. 456
Further Studies on the Effect of Sulfanilamide on Experimental Tuberculosis. Richard H. Follis, Jr., and Arnold R. Rich 466
Nerve Endings in the Cardiac Muscle of the Rat. Arthur B. King 489
Book Reviews 500
Index to Volume LXV 505

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BULLETIN
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FRANK R. SMITH

UNIV. OF MICH.

Frank Robert Smith
1856—1938

The first number of *The Bulletin of The Johns Hopkins Hospital* was published in December 1889. During the half century which has passed, one man did more than any other to sustain the editorial precepts which were established under Henry M. Hurd, the first editor. This man was Frank R. Smith.

For almost fifty years Dr. Smith served in the capacity of typographical editor: correcting and rearranging manuscripts, reading all galley- and page-proof, interviewing authors, and personally delivering all copy to the printer. This work was his hobby and he devoted a great amount of time to it. Even after he had reached an age at which he found it necessary to limit his large medical practice, he continued energetically to perform his editorial work. In the early days of his final illness he completed the preparation of the page-proof of the September 1938 number. His death on September 26, 1938 took from the Editorial Board its most experienced, most active and most loyal member.

At the time of the celebration of the fiftieth anniversary of the hospital, May 5, 1939, a tablet was presented to the hospital "To commemorate the services of Frank R. Smith, M.D., B.A., M.A.— A loyal member of the staff of The Johns Hopkins Hospital and Medical School from 1891 until his death." The Editorial Board takes this occasion to publish the presentation address which was delivered by Dr. Thomas S. Cullen.

Presentation of a Tablet to the Memory of Dr. Frank R. Smith

Frank R. Smith was born on June 19, 1856, in Carlton-Carville, near Lowestoft, Sussex, England. He was the thirteenth child of Dr. and Mrs. Samuel Smith.

Frank Smith was educated in various schools, largely through scholarships. Most of them were church schools where he was required to sing in the choir. His family hoped that he would become a minister. He went to Cambridge University, Saint Catherine's

College, where he graduated in 1884. He majored in Latin and Greek. Later he joined a brother in Grand Forks, North Dakota, where, for a time, he was a book-keeper for his brother.

Smith finally decided to study medicine. His first two years were spent at Rush Medical College in Chicago, his last year at the University of Maryland. He graduated in 1891. During his student days here he tutored two boys, one of whom later became prominent in Baltimore life.

In 1891 Smith became an assistant-resident in medicine in The Johns Hopkins Hospital. When I came on the staff in January 1892 Frank Smith was a very important figure in our lives. The thought of a Johns Hopkins Medical School was in the dim distance. Residents, assistant-residents and interns were selected from all parts of the United States and Canada.

Frequently after a six o'clock supper we would go to Frank Smith's room. Among those present were Billy Thayer, Lew Barker, Billy Russell, John Hewetson, August Hoch, Albert Stavely, Parker, Simon Flexner and George Nuttall from the Laboratory, and a few others. Frank Smith would read aloud *Tom Sawyer*, *Huckleberry Finn*, or some other interesting book for an hour, and we would then disperse to make rounds in various parts of the hospital. Even in those early days Smith was much beloved by his fellow staff members.

Dr. Osler, with his clear insight into men, soon appreciated Smith's phenomenal knowledge of the English language, and had him correct the English of at least one edition of his *Practice of Medicine*. Many of the junior and senior men sought his advice when publishing monographs or books. During the last 45 years Frank Smith has gone over the English of every article I have published. It was truly remarkable how, by a slight change here and a little alteration there, he would transform a poor paper into a respectable one.

After Smith started in practice he still retained his connection with the medical department of the hospital and when he rested from his labors on September 26, 1938, he was Associate Emeritus in Clinical Medicine in The Johns Hopkins University and Assistant Visiting Physician to The Johns Hopkins Hospital.

Frank Smith not only gained much inspiration from the hospital but he also became deeply interested in one of the nurses of The Johns

Hopkins Hospital and married Anna C. Jack, a valued graduate of the training school.

Frank Smith had a large practice throughout his long years of service. He was not only the patient's physician but also his dear friend, and his patients idolized him. How many of us have traveled along in an open street-car on Caroline Street and heard two women discussing the condition of an ill one in their family. The first woman would describe the illness, the second one would ask, "Who is your doctor," and on receiving an answer would reply, "You must see my doctor, Professor Frank Schmidt, at once." It gave one a feeling of well-being to realize that our friend had such an abiding place in the hearts of his patients.

Dr. and Mrs. Smith had two children, Dorothea and Frank, Jr. Both gave him great joy. Frank, Jr. has followed in his father's footsteps. He is Instructor in Medicine in the medical school and is an Assistant Visiting Physician to The Johns Hopkins Hospital.

Frank Smith was one of the most simple and unassuming men I have ever met. He was the soul of honor and had hosts of friends. He had a merry twinkle in his eye and a rare sense of humor. Occasionally he would unbend, especially when we reminded him of how near he came to being a clergyman or "sky pilot." During his student days in the West he had actually written sermons for a clergyman who was not gifted in preaching.

Smith worked right up until near the last. Only a few weeks before his passing one would see him carefully driving his car on his way to see his patients. For years he had been one of the editors of the Bulletin of the Johns Hopkins Hospital. When taken ill he had already prepared the galley- and page-proof for the September number, and before October he was gone.

The family of Dr. Smith have had a memorial tablet made by Henry Berge, the sculptor, and this is now to be presented to The Johns Hopkins Hospital. I deem it a great honor to have been asked by the family to make this presentation.

On behalf of Mrs. Frank R. Smith, Mrs. Max Ways, and Dr. Frank R. Smith, Jr., I have the rare privilege of presenting this tablet to The Johns Hopkins Hospital, where Frank R. Smith spent so many useful and happy years.

ELECTROCARDIOGRAPHIC CHANGES IN ACUTE HEMORRHAGIC NEPHRITIS

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Submitted for publication July 29, 1939

The occurrence of cardiac failure in acute nephritis, although recognized many years ago, has but recently attracted much attention. Manifestations of myocardial dysfunction, if carefully looked for, are to be found in a surprisingly large proportion of even the milder cases. One of these manifestations, the changes observed in electrocardiograms, has been stressed in several recent contributions to the subject (1, 2, 3, 4). A summary of these changes together with those reported here is given in Table I.

Langendorf and Pick (1), studying 10 cases of acute nephritis, have found an electrocardiographic picture somewhat similar to that seen in the later stages of infarction of the anterior surface of the heart. This picture consisted of flattening or inversion of T_1, together with a large upright T_3, and a biphasic or inverted T wave in the precordial lead. These changes were considered by the authors to have some degree of specificity as they occurred to a greater or lesser extent in 9 of their 10 cases. No exact correlation was observed between the electrocardiographic changes and the degree of cardiac or renal insufficiency.

Masters, Joffe and Dock (2) reported 24 cases, 10 of which followed the pattern just described, save that they did not show the elevated T waves in lead 3. Four cases showed high pointed T waves, 4 rather low T waves, and in 5 the P-R interval was prolonged. Tur (3) is said to have reported similar T wave changes. Five cases showed a negative T wave in the precordial lead which was always associated with inversion of T waves in the standard leads, and in 3 cases the initial positive deflection was absent, remaining small in two. These authors were inclined to consider the changes non-specific and merely

indicative of myocardial dysfunction. Again no very exact correlation could be demonstrated between the cardiac and renal signs and the electrocardiographic changes.

Garreton Silva, Hervé, Nogues and Forero (4) reported 36 cases, in 18 of which flattening or inversion of T_1 and T_2 occurred. They also found prolongation of Q-T interval in 11 cases. They believed, contrary to the conclusions of others, that the degree of electrocardiographic change could be fairly accurately correlated with the clinical evidence of heart failure.

The present report deals with 15 cases of acute glomerular nephritis observed during the past two years. These cases form part of a

TABLE I

AUTHOR	LANGENDORF AND PICK (10 CASES)		MASTERS ET AL. (24 CASES)		GARRETON SILVA ET AL. (36 CASES)		PRESENT SERIES (15 CASES)	
	Number of cases	Per cent	Number of cases	Per cent	Number of cases	Per cent	Number of cases	Per cent
Abnormalities of P. wave	—	—	4	17	5	14	0	0
Prolonged P-R interval	0	0	5	21	0	0	2	13
Slurring of QRS	—	—	6	25	22	62	1	7
T waves high and pointed	—	—	4	17	—	—	—	—
T waves low	2	20	4	17	—	—	4	27
T waves isoelectric or inverted	9	90	10	42	18	51	3	20
Absent initial positive deflection in chest lead	—	—	3	13	—	—	0	0
T wave inverted in chest lead	3	30	5	20	—	—	5	33

larger group in which it was demonstrated that the severity of the myocardial failure was in most cases roughly proportional to the severity of the nephritis (5). All were carefully followed from clinical and laboratory standpoints. Serial electrocardiograms were taken at intervals of 2 to 3 days during the acute stage, in 11 cases between the 2nd and 8th day of disease and at somewhat less frequent intervals during convalescence.

The most striking changes were in the T waves. In three cases the T waves in the standard leads were isoelectric or inverted, one showing the changes primarily in leads 1 and 2, one simulating a posterior rather than an anterior infarction, and the third showing T wave

TABLE II

CASE NO.	AGE AND SEX	DAY OF DISEASE	BLOOD PRESSURE	VENOUS PRESSURE	DIAMETER OF HEART BY X-RAY	WEIGHT	NPN	PSP	HEMATURIA RBC/HPF	ALBUMINURIA, GMS./LI.	ELECTROCARDIOGRAPHIC FINDINGS
1	18	35	115/60	120	10.4	132	25	70	10	4	T 4 biphasic. Standard leads normal
I. B.	F	39	96/60	80		110	24		1–2	0.5	No change. Changes probably not significant
		55	100/60		10.2				0	0	
2	19	5	115/70	105	12.8	165	24	90	10	2	P-R interval 0.22. No other changes
B. W.	F	9	120/70			135			1–2	0.25	P-R interval 0.16. 4 subsequent records normal
		25	130/70		11.6	127	24		0	0	
3	26	2	125/70	65	11.7	150	34		30	0.25	T 4 biphasic. Standard leads normal throughout
W. D.	M	4	110/55	65			33		75	0.25	T 4 slightly biphasic
		6	125/70				50		150+	1.5	T 4 upright
		9	118/64				42		150+	0.25	T 4 upright but low
		16	100/50	80	11.9	150	30	85	0	0	T 4 slightly biphasic. 3 subsequent records showed a normal T 4
4	42	21	190/110	85	14.7	142	34	70	15	3	Normal records throughout, save for premature beats of nodal
J. G.	M	26	140/80				34		5	3	origin, which were still present 7 months later
		31	110/70		13.5	135			10	2	
		41	110/70		13.0	132	40	65	1	0	
5	35	45	150/90	65	14.9	161	36	75	5	0.2	T 1 normal. T 2 and 3 inverted. T 4 high and peaked. Q-T
J. T.	M	60	130/80			148		80	10	0.2	interval slightly prolonged
		75	120/75		12.7		30		10	0.2	T 2 flat. T 3 less deep. T 4 less high. Q-T interval normal
		86	120/75		13.0	146			2	0.2	T 2 slightly inverted. T 3 deeper and cove-plane
		100	120/75						2	0.2	T 2 upright but low. T 3 less deep
		111	120/75		13.4	146			2	0	Essentially a normal record
6	13	5	120/70	120	9.9	91	28	35	75	3.0	Standard leads normal throughout. Sinus tachycardia. 6
D. R.	F	12	110/70	90		88	34		50	0.5	records between the 5th and 29th days showed T 4 sometimes
		29	105/70			89		80	15	trace	biphasic or inverted, sometimes upright and low
		33	120/80						5	trace	T 4 inverted
		39	120/80		9.3	92	30	80	25	trace	T 4 more sharply inverted
		52	120/80			88		95	10	trace	T 4 upright. 7 subsequent records show T 4 always upright, although sometimes rather low

7	30	7	155/105	135	12.2	127	39	70	150+	trace	Right axis deviation. T 1 almost flat. T 4 inverted
W. W.	M	12	140/95	80		113			15	trace	Normal axis. T 1 upright but low. T 4 upright
		18	140/100		9.8	111			10	trace	T 1 remains low. Record otherwise normal
		27	130/85						10	trace	Normal record. 5 subsequent records were normal
		40	125/85		10.5	109	30	75	5	trace	
8	43	15	175/90	75	16.3		38	70	10	1.0	Standard leads normal. T 4 inverted. Low initial positive deflection in chest lead
G. B.	F										
		26	130/85		14.3	166	26	75	10	0.5	Normal record.
		41	130/85			165			40	0.5	5 subsequent records were normal. This patient had received digitalis prior to her illness, but had had none for the previous 3 weeks before admission
		100	130/85		12.8	166	30	80	5	0	
9	15	3	135/80	90	12.5		100		2	0.2	Normal record. Received 0.2 gram of digitalis on the 4th and 5th days. Records on the 5th, 11th, and 18th days were perfectly normal
H. C.	M	6	120/70				128	40	5	0	
		13	110/55	75	13.2		60		5	0	
		20	110/75				38	65	5	0	
		35	110/75				30	75	2	0	
10	17	3	150/95	160	13.4	144	58	70	150−	0.2	Standard leads normal. T 4 biphasic
J. G.	M	5	145/105			148	44		15	0.2	T 2 low. T 4 inverted
		7	135/95	145		142	48		0	0	T 2 almost flat. T 4 now upright but low
		10	140/95	125	11.9	136	35		5	0	Digitalis given from the 8th to the 11th days. Subsequent records showed low or flat T waves, but there was no inversion. Records normal by the 31st day and thereafter
		18	120/70	90	10.8	129		75	2	0	
		31	120/70		10.9	127		70	4	0	
11	34	6	155/80	180		141	32	90	50	2.0	Normal record
J. B.	M	8	135/85	155		136	26		80	0.5	T 2 low
		11	115/70	100					50	1.0	T 2 low
		15	115/70			126	38	90	20	2.0	T 2 normal
		25	115/70			129	38		20	2.0	T 2 low
		30	115/70			130			150	2.0	T 2 normal. 5 subsequent records showed normal complexes
		70	115/70			137	36	75	10	trace	
12	16	5	170/110	145	11.8	143	40		30	3.0	Normal record. Digitalis from the 6th to the 40th days. Records during this period showed only lowering of the T waves. No inversion save in the chest lead. On the 14th day marked right axis deviation was present. This had disappeared by the 20th day. Records after the 60th day were normal
E. C.	M	7	145/90	95				80	15	0.2	
		12	160/110	75	11.3		42		20	3.0	
		14	160/115			132	50		15	0.5	
		20	165/100		11.3	129	50	70	150	2.0	
		51	145/85			125	33	85	5	trace	
		91	125/70			142			2	0	

TABLE II—*Concluded*

CASE NO.	AGE AND SEX	DAY OF DISEASE	BLOOD PRESSURE	VENOUS PRESSURE	DIAMETER OF HEART BY X-RAY	WEIGHT	NPN	PSP	HEMATURIA RBC/HPF	ALBUMINURIA, GMS./LI.	ELECTROCARDIOGRAPHIC FINDINGS
13 C. M.	60 M	6	180/95	225	14.9	225	32	60	100	2.5	Normal record. Digitalis from the 7th day on. Subsequent records showed lowered T waves and sagging S-T intervals. No inversion of the T waves
		12	165/100	85		215	32		10	1.0	
		24	130/80		14.1	186	60	70	5	1.0	
		75	130/80		12.5	176	34		1	0	
14 H. W.	30 M	7	150/105	160	15.5	170	28	55	10	1.0	P-R interval 0.21. T waves normal
		10	150/100	160					10	0.2	P-R interval 0.18. T waves normal
		13	155/100	115		155			5	trace	P-R interval 0.18. T 1 and 2 slightly low
		16	135/80	140		150			2	0	P-R interval 0.18. T 1 and 2 somewhat lower
		23	120/80	60	11.3			55	rare	0	P-R interval 0.16. T 1 low. T 2 isoelectric
		30	120/80			145	44		0	0	P-R interval 0.18. Normal record
15 W. J.	36 M	6	120/70	130	14.8		216		150+	2.0	Normal record. Patient received 1.0 gram of digitalis on the 7th day but none thereafter. From the 8th to the 22nd day the initial deflection in the chest lead was low, often less than 1.0 mm. T 1 became low and T 2 inverted. In view of the nature, degree and duration of these changes it is doubtful if they represent digitalis effect
		8	150/90	130			196		25	1.0	
		12	160/90		16.4	196	140	25	10	2.5	
		17	160/100	130			52		10	3.0	
		24	125/80	75	16.0	180			3	1.5	
		28	125/80	75	14.2		34		1	0.5	Initial positive deflection higher. T 1 biphasic. T 2 and 3 inverted
		40	125/80		14.4	173	35	70	20	0.5	Initial positive deflection normal. T 1 up but low. T 2 and 3 inverted
		55	125/80		13.8	170	42	55	40	0.5	T 1 up but still low. T 2 isoelectric. T 3 less deeply inverted
		67	125/80		13.8	172		45	30	0.2	T 1 normal. T 2 upright but low. T 3 isoelectric
		80	125/80		12.8	173		75	15	trace	Normal record

depression and inversion in all the standard leads. Four other cases showed lowering of the T waves in leads 1 and 2 of varying degree but sufficient to be significant. Five cases with no alterations in the standard leads showed biphasic and inverted T waves in the precordial lead (CF3). Repeated subsequent normal records, together with a gradual recession of the changes sometimes observed in the serial records served to rule out technical errors as a source of these changes. In 2 cases the initial positive deflection in the precordial lead was low, subsequently returning to normal, and in 2 cases there was a temporary increase in the duration of the P-R interval to 0.21 and 0.22 seconds. In the 3 cases showing left axis deviation this was a constant finding; 2 cases, however, showed a transient right axis deviation. The Q-T interval was constantly prolonged in 1 case and transiently prolonged in another.

The number of cases studied is too small to permit accurate deductions in respect to any possible correlation between the electrocardiographic changes and the extent of the myocardial and renal insufficiency. In general there was no apparent correlation. Of the 5 cases classed as severe nephritis, all of which showed clinical evidence of heart failure, only two had electrocardiographic changes of more than slight degree. (Table II, cases 14 and 15. Also Figs. 2 and 3.) Among the 5 moderately severe and 5 mild cases there was a complete scattering of electrocardiographic changes; and the cases which did show changes, had in general no more signs of myocardial insufficiency than the others. A striking feature, which has been commented upon by other authors, is the fact that the electrocardiographic changes sometimes reach their maximum after the acute stage of the disease is over, when the signs of renal insufficiency or damage are much diminished, the heart shadow smaller in size, and the blood pressure lowered. The changes may then persist with some modification for as long as several weeks before the pattern gradually reverts to normal. In our experience the changes have not been so specific as Langendorf and Pick assumed, and have been, on the whole, much less striking.

It is generally agreed that the changes in the size and shape of the heart are not responsible for the electrocardiographic findings encountered, and the fact that such findings may persist long after the

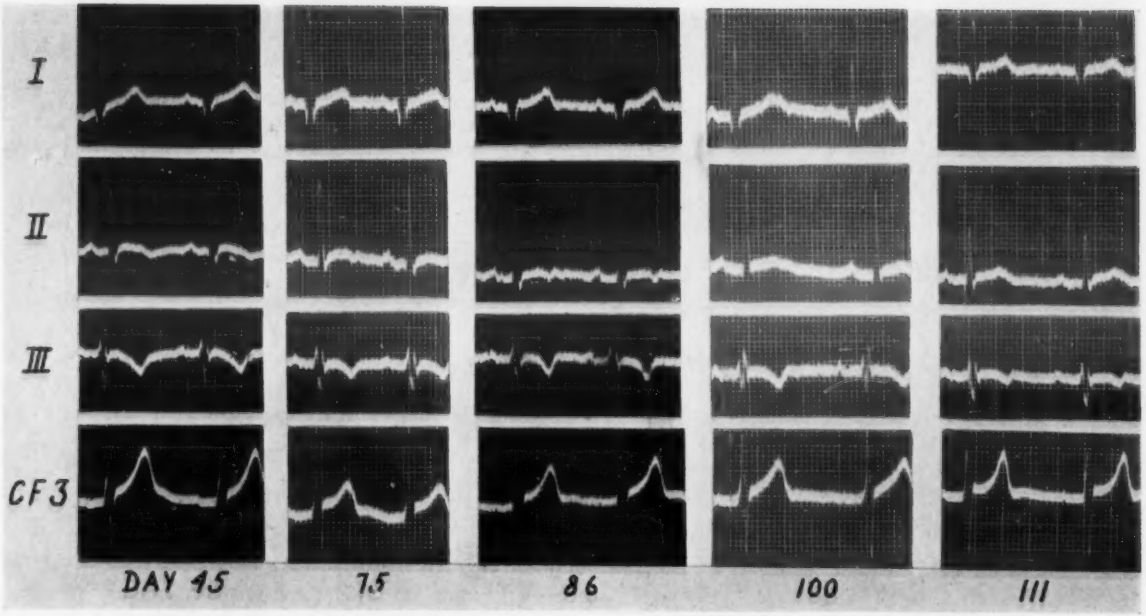

FIG. 1. SERIES OF ELECTROCARDIOGRAMS TAKEN IN CASE 5, 45 TO 111 DAYS AFTER THE ONSET OF ACUTE HEMORRHAGIC NEPHRITIS

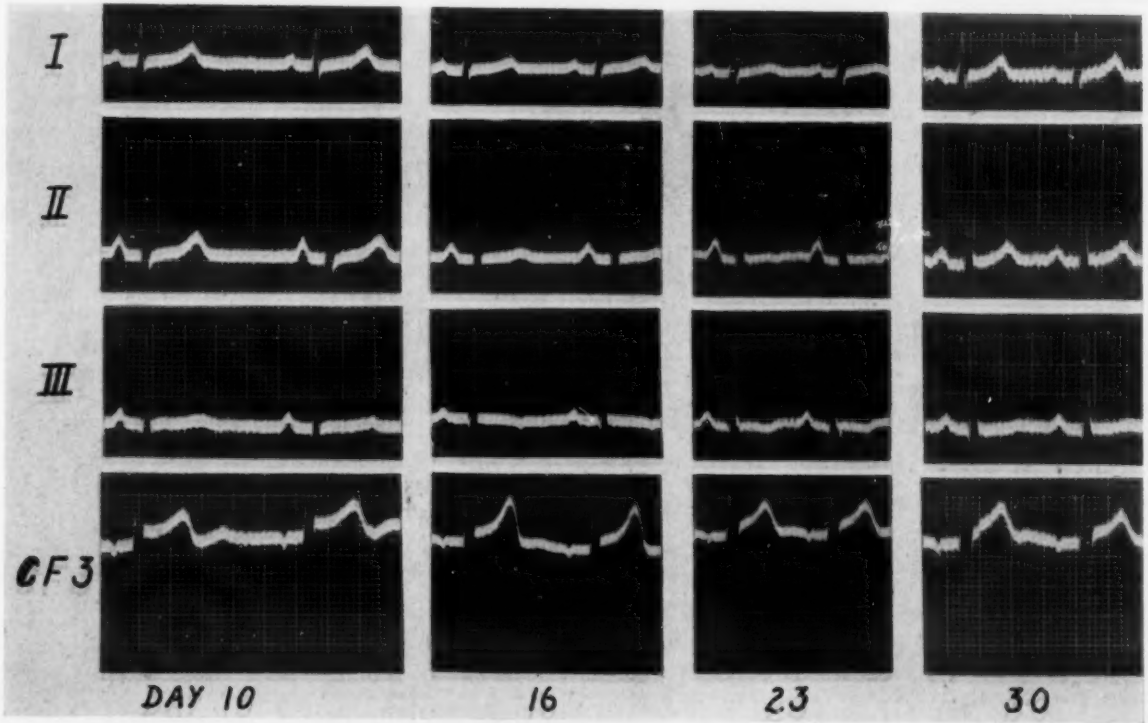

FIG. 2. SERIES OF ELECTROCARDIOGRAMS TAKEN IN CASE 14, 10 TO 30 DAYS AFTER THE ONSET OF ACUTE HEMORRHAGIC NEPHRITIS

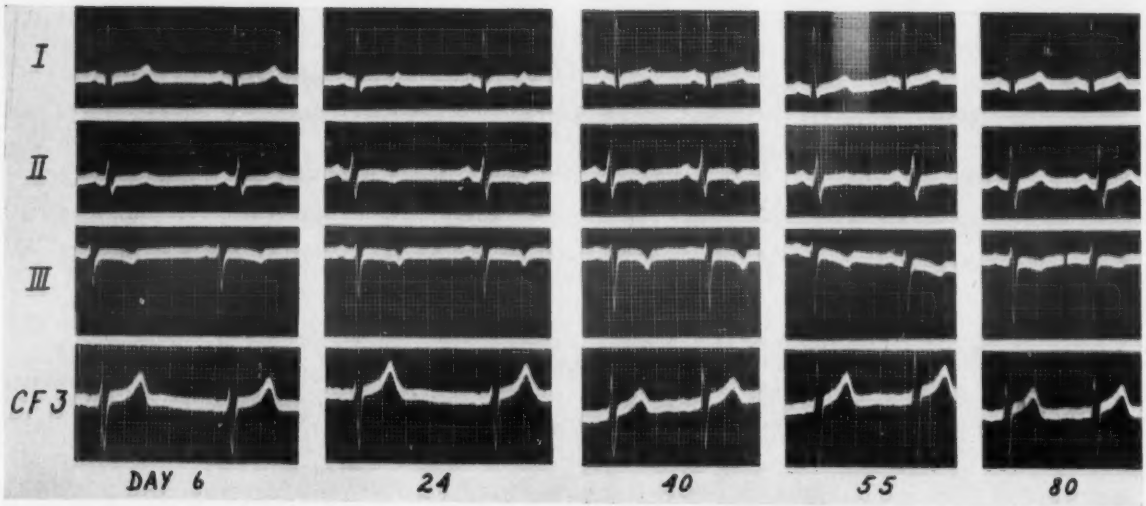

FIG. 3. SERIES OF ELECTROCARDIOGRAMS TAKEN IN CASE 15, 6 TO 80 DAYS AFTER THE ONSET OF ACUTE HEMORRHAGIC NEPHRITIS

teleoroentgenograms have returned to normal would seem to bear this out. The most logical conclusion that can be drawn is that the changes indicate a functional derangement of the myocardium, at times widespread, and of unknown etiology. The most attractive hypothesis in this regard is that the disease is essentially one of widespread capillary damage which may affect the vessels of the myocardium more or less severely.

In an effort to elucidate some of these questions an attempt was made to determine whether such electrocardiographic changes could be reproduced in animals. Rabbits in which acute glomerular nephritis had been induced by the method of Masugi (6) and which were being used in the course of other experiments, were followed throughout their illness with serial x-rays and electrocardiograms. The clinical course of the acute nephritis in the animals studied was quite similar to that seen in humans, except for the fact that the changes in blood pressure were comparatively slight (7). Thirty-seven electrocardiograms were taken on 11 rabbits, and 19 x-rays on 6 rabbits, but no significant changes could be demonstrated.

SUMMARY

Fifteen cases of acute glomerular nephritis have been followed with serial electrocardiograms. Changes were observed in 10 cases and consisted chiefly in flattening and inversion of the T waves in one or more leads, including the precordial lead. Changes were noted less frequently in the initial positive deflection in the precordial lead, and in the auriculo-ventricular conduction time. No correlation could be established between the occurrence of these changes and the signs of cardiac and renal insufficiency, but the series is too small to permit accurate deductions in this respect. Rabbits in which acute nephritis had been induced by the method of Masugi, failed to show comparable changes in their electrocardiographic patterns.

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CYANOSIS IN CHICKENS AND MICE INDUCED BY SULFANILAMIDE

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Cyanosis was noted in the earliest series of patients treated with sulfanilamide and has proven to be the most common complication accompanying therapy with this drug. In spite of many clinical and experimental studies the exact origin of the dark colored blood has not been clearly established. In the earliest English reports (1) sulfhemoglobin was described, while later reports emphasized the importance of methemoglobin (2). Marshall and Walzl (3) in a study of bloods from a series of cyanotic patients in which they compared oxygen capacity with total blood iron concluded that "the cyanosis may not be due to non-functional iron pigment," and suggested that the dark color might in part be due to an oxidation product of the drug which stained the red cells. Chesley (4) using Marshall and Walzl's technique, and Evelyn and Malloy (4a) using a colorimetric method for the estimation of blood pigments, also came to the conclusion that the formation of methemoglobin or sulfhemoglobin could not account for the dark colored blood. Ottenberg and Fox (5), discovering that dilute solutions of sulfanilamide exposed to ultra violet light turned purple, suggested that some such pigment might account for the cyanosis. However, there is at present no direct evidence that the pigment they described plays any rôle in the development of the cyanosis. Wendel (6), Hartmann, Perley and Barnett (7), and Watson, Vigness and Spink (8) relying chiefly on spectroscopic studies believe that methemoglobin or sulfhemoglobin is present in sufficient quantity to account for nearly all the dark colored blood. Webb and Kniazuk (9) using a spectrophotometer have reported that repeated

[1] National Research Council Fellow in Medicine, 1938–1939.

[2] The sulfanilamide used in this study was furnished by Dr. K. K. Chen of Eli Lilly and Company.

administration of large doses of sulfanilamide to rats resulted in the formation of methemoglobin, sulfhemoglobin, and some unidentified pigment. Similar spectrophotometric studies by Wendel (10) and Watson, Vigness and Spink (8) in human patients have failed to find the unidentified pigment of Webb and Kniazuk.

At the present time it is agreed by everyone who has worked in the field that methemoglobin is formed in appreciable quantities by many patients under therapy with sulfanilamide. There is not however complete agreement as to whether non-functional iron pigments can account for the entire discoloration. Difficulty in settling this question is due to three factors:

(1) There is no exact method of determining the presence or absence of cyanosis or of estimating its degree. It is important in studies of this nature that cyanosis should be determined by the examination of drawn, aerated blood, and not on the appearance of the patient. If possible, the degree of discoloration should be established by comparison with some fixed standard, or to blood to which a known concentration of methemoglobin has been added.

(2) Conclusions have oftentimes been based on incomplete or unsatisfactory evidence. In order for cyanosis to be explicable by any particular abnormal pigment, it is necessary that the pigment be identified qualitatively, determined quantitatively, and then it must be shown that the color of the cyanotic blood matches normal control blood to which an equivalent amount of abnormal pigment has been added.

(3) Up to the present time it has been difficult to produce an experimental cyanosis under such conditions in animals that it could be studied satisfactorily.

Within the past year we have been able to produce a cyanosis in chickens, pigeons, and mice, and wish to present in this paper results of blood studies in these animals.

METHODS

For the identification of abnormal blood pigments each sample of blood was examined spectroscopically with a Wood replica grating spectrometer, illumination being furnished by a sixty-watt electric light bulb. Whole blood was hemolyzed with solid saponin and diluted gradually until the region in the red from 600 to 700 $m\mu$ was visible or until a definite band could be seen. The usual tests using

cyanide, hydrosulfite, and carbon monoxide were applied to any blood giving a band in the red in order to further identify it.

Active hemoglobin was determined by the gasometric methods of Van Slyke (11). In most cases oxygen capacity was determined, but in some instances carbon monoxide capacity was also estimated. Determinations were always done in duplicate.

Because of the possible presence of unidentified pigments, and of abnormal hemoglobin derivatives, determination of total blood pigment by the colorimetric methods of Sahli, Newcomer, and others, are unsatisfactory. For these experiments total hemoglobin was estimated by determination of total blood iron. The method of Coombs (12), utilizing the dipyridyl color reaction after a digestion of the blood with sulfuric and nitric acids, was used. Final readings were made with a photoelectric colorimeter and expressed in terms of volumes per cent oxygen. Determinations were done in quadruplicate.

The difference between oxygen capacity and blood iron gave non-functional iron pigment regardless of its nature. Normal blood containing an equivalent amount of methemoglobin was then prepared, and if the resulting mixture matched the experimental blood in color it was assumed that cyanosis was due to non-functional iron pigment. The presence of methemoglobin in cyanotic blood was further tested for by adding a few crystals of sodium cyanide to the whole blood. If the dark color disappeared, or nearly so, it was assumed that the cyanosis was likely due to methemoglobin. Blood sulfanilamide concentration was determined by Marshall's method (13) using a special photoelectric colorimeter (14) for the final readings.

CYANOSIS IN CHICKENS

These studies were started following the observation by Litchfield (15) that the administration of large doses of sulfanilamide to chickens resulted in a marked cyanosis. A total of 24 chickens of mixed breed and sex, fed a diet of whole cracked grain, were used. In a few instances dosage was in the form of a 1 per cent solution by mouth, also intramuscularly, but as it became apparent that mode of administration made no difference as long as absorption took place, it was found most convenient to give the drug by mouth in capsules.

The daily administration of 0.5 to 2.0 gms. sulfanilamide per kilogram invariably produced an intense cyanosis within at least 4 to 5 days. With doses of 1.0 to 2.0 gms. per kilogram symptoms often became evident within 24 to 36 hours. The bird's comb became dark brown, mucous membranes were discolored and drawn, aerated blood was markedly dark in color. In addition, most of the birds showed

marked systemic symptoms ranging from a slight ataxia to coma, and a condition which resembled decerebrate rigidity. Hyperpnea was almost never observed even though it was shown later that as much as 40 per cent of the hemoglobin had been inactivated.

Table 1 presents a summary of detailed studies on nine of these birds. The difference between total iron pigment and active hemoglobin divided by the value for total iron pigment is presented in the table as per cent inactive iron. The results show that in every in-

TABLE 1

Sulfanilamide Cyanosis in Chickens

BIRD NUMBER	DOSE SULFANILAMIDE	BLOOD LEVEL		DARK COLOR BLOOD	INACTIVE IRON		SPECTROSCOPE METHEMOGLOBIN
		Free	Total		Before sulfanilamide	After sulfanilamide	
	gms./kilo per day	*mg. per 100 cc.*	*mg. per 100 cc.*		*per cent*	*per cent*	
7	2.0 × 3	117.	118.	+++	1.0	21.8	
8	2.0 × 4	28.4	36.6	+++	2.5	20.9	
9	2.0 × 4	20.6	44.8	+++	2.7	44.8	
23	2.0 × 2	29.6	43.8	+++		27.4	(+)
24	2.0 × 3			+++			(+)
11	1.0 × 1	21.4	34.2	++	0.0	12.5	
	1.0 × 9	54.0	72.0	+++	0.0	24.4	(+)
12	1.0 × 2	76.4	80.4	(−)	3.7	3.8	(−)
15	0.5 × 6	60.4	74.8	++	5.9	21.0	(+)
17	0.5 × 7	27.6	40.0	+++	0.5	27.9	(+)
1	0.5 × 11	94.8	104.8	+++		42.9	(+)
21*	2.0 × 4	28.0	45.5	++		26.0	(+)
22*	2.0 × 3	46.5	56.0	+++		27.0	(+)

* These two animals received acetylsulfanilamide.

stance in which cyanosis was present the amount of non-oxygen carrying blood pigment was markedly increased. In every cyanotic blood which was examined spectroscopically a band was observed at 630 mμ. Addition of cyanide and hydrosulfite caused the band to disappear readily while carbon monoxide had no effect on it. The addition of a few crystals of sodium cyanide to the whole cyanotic blood caused it to turn a bright red which nearly matched the color of normal chicken's blood. These reactions are all characteristic of methemoglobin.

Following the determination of inactive hemoglobin an equivalent amount of preformed methemoglobin was added to normal control blood. In every instance the result was a cyanotic blood which closely matched the blood obtained from the chicken receiving sulfanilamide. It therefore seemed certain that in this series of birds the sulfanilamide cyanosis could be explained entirely by the formation of methemoglobin.

Two chickens identified in the table by asterisks received acetylsulfanilamide, 2.0 gms. per kilogram daily. Both became cyanotic and examination of the blood showed the presence of sufficient methemoglobin to account for the discoloration. However, when the sulfanilamide content of the blood was determined, it was seen that most of the acetylated compound had been converted to the free form. These two birds therefore in themselves give no information as to whether the acetylsulfanilamide by itself is effective in forming methemoglobin.

In a limited number of experiments, 12 pigeons were given daily doses of 1.0 and 2.0 gms. sulfanilamide per kilogram. Although cyanosis and methemoglobinemia were produced, a much longer period of time was required, and in no instance were the changes as marked as those observed in chickens.

CYANOSIS IN MICE

A regular strain of Swiss mice bred in this laboratory for chemotherapeutic studies was used. The diet consisted of finely ground Purina Dog Chow into which appropriate concentrations of sulfanilamide were thoroughly incorporated. The mice were allowed to feed *ad lib*. Under the conditions of the experiment they ate regularly throughout the twenty-four hours and thus maintained a fairly constant level of drug in the blood. Detailed studies on this method of administering chemotherapeutic agents is being published elsewhere (16).

Groups of mice were fed diets containing from 0.25 per cent to 2.0 per cent sulfanilamide. Cyanotic blood was observed in all groups of animals, although irregularly on the lower concentrations. Cyanosis usually became evident in from 3 to 5 days, and for the purposes of the studies reported here medication was continued for seven days.

At the end of this time mice on the higher concentrations of the drug presented a striking picture. Eyes, tail, and mucous membranes were all discolored, and drawn aerated blood was markedly dark in color. Animals seldom showed symptoms of intoxication other than ruffled fur and a slight loss of weight. On the lower concentrations of the drug cyanosis was less striking and might have been missed if the animals had not been compared with normal controls.

TABLE 2

Sulfanilamide Cyanosis in White Mice

NUMBER OF MICE	PER CENT SULFANILAMIDE IN DIET	SULFANILAMIDE CONSUMED	BLOOD LEVEL		OXYGEN		INACTIVE IRON	DARK COLOR BLOOD	SPECTROSCOPE SULFHEMOGLOBIN
			Free	Total	Blood iron	Oxygen capacity			
		gms./kg./day	*mg. per 100 cc.*	*mg. per 100 cc.*	*volumes per cent*	*volumes per cent*	*per cent*		
12	0.0	0.0	0.0	0.0	18.0	18.3	−1.6	(−)	(−)
6	0.0	0.0	0.0	0.0	17.6	17.67	−0.4	(−)	(−)
6	0.0	0.0	0.0	0.0	17.2	17.45	−1.4	(−)	(−)
6	0.0	0.0	0.0	0.0	18.2	17.6	3.2	(−)	(−)
4	0.25	0.38	1.3	2.1	18.1	15.4	15.4	(+)	(+)
5	0.25	0.38	1.7	2.5	12.6	11.7	7.1	(±)	(±)
5	0.25	0.39	1.7	2.5	16.8	15.11	10.6	(+)	(+)
7	0.5	0.99	2.2	4.0	17.2	15.7	8.7	(+)	(+)
7	0.5	0.92	2.3	3.8	16.1	14.5	10.1	(+)	(+)
7	0.5	0.92	4.2	6.2	14.8	13.8	8.1	(+)	(+)
6	1.0	1.37	9.0	11.2	17.3	14.5	16.1	(++)	(+)
6	1.0	1.37	8.8	11.0	16.0	13.8	13.7	(++)	(+)
6	1.0	1.20			14.6	12.8	12.3	(++)	(+)
10	1.0		5.6	8.0	17.6	16.4	8.0	(+)	(+)
6	2.0	2.33	12.4	17.2	16.3	14.1	13.9	(++)	(+)
6	2.0	2.33	24.0	26.0	16.2	13.1	19.4	(+++)	(+)
12	2.0		14.0	17.0	15.6	12.7	18.3	(+++)	(+)
6	2.0	2.66	11.9	14.2	14.6	11.9	18.5	(+++)	(+)

Because of the quantity required, blood from several mice had to be pooled for one set of determinations. It was thus obviously not practical to do control studies on mice before treatment, and for this reason results obtained with this species had to be compared to other mice which had received no sulfanilamide. Blood was obtained by cardiac puncture and the composite sample kept liquid by the addition of a small amount of oxalate.

Table 2 presents a summary of results obtained from a study of 123

mice. Per cent inactive iron was calculated as described above. Every cyanotic blood examined spectroscopically showed a band at 620 mμ which was uneffected by cyanide or hydrosulfite, but which shifted 5 to 10 mμ to the left when carbon monoxide was bubbled through the sample. Crystals of sodium cyanide added to the whole cyanotic blood changed the color very little. These reactions are all characteristic of sulfhemoglobin.

Table 3 presents results on a group of mice which were fed 1 and 2 per cent acetylsulfanilamide. As can be seen from the figures cyanosis and sulfhemoglobin were produced consistently. In general the results were less striking than those obtained with similar dosage of

TABLE 3

Cyanosis Following Administration of Acetylsulfanilamide to White Mice

NUMBER OF MICE	SULFANILAMIDE IN DIET	SULFANILAMIDE IN BLOOD		INACTIVE IRON	DARK COLOR BLOOD	SPECTROSCOPE SULFHEMOGLOBIN
		Free	Total			
	per cent	*mgm. per cent*	*mgm. per cent*	*per cent*		
6	1.0	0.6	4.7	14.9	++	(+)
6	1.0	0.8	5.2	10.6	+	(+)
6	1.0	1.2	4.2	14.2	++	(+)
7	2.0	0.6	6.2	14.4	++	(+)
7	2.0	1.0	9.7	12.8	+	(+)

sulfanilamide. Blood level determination revealed, however, that this could be explained on the basis of poorer absorption of the drug.

The results presented here indicate that cyanosis in mice under conditions described is explicable on the basis of the formation of a non-functional iron blood pigment which gives the recognized reactions of sulfhemoglobin.

ACTION OF SULFANILAMIDE ON BLOOD IN VITRO

That sulfanilamide is effective in converting hemoglobin into methemoglobin in the test tube can be shown by the following experiments. Tubes of washed human red cells containing 10, 50, and 100 mgms. of sulfanilamide were incubated at 37, 39 and 41 degrees centigrade for 24 to 48 hours. At the end of this time all bloods were examined spectroscopically for the formation of abnormal blood pigments.

At 37°C. formation of methemoglobin was uncertain and usually small even with 100 mgm. per cent of the drug. At 39° and 41° the reaction was more regular and in the presence of 100 mgms. per cent of sulfanilamide; as much as 30 per cent of the hemoglobin was converted to methemoglobin at 41° in some of the experiments. Controls incubated at the same temperature showed only oxyhemoglobin. It is important that washed corpuscles be used, as the presence of reducing substances in plasma tend to prevent the oxidation of hemoglobin from going forward.

TABLE 4

The Effect of Methylene Blue on Sulfanilamide Cyanosis in Chickens

BIRD NUMBER	DOSE OF SULFANILAMIDE	BEFORE METHYLENE BLUE		AFTER METHYLENE BLUE	
		Dark color blood	Inactive iron	Dark color blood	Inactive iron
	gms./kilo per day		*per cent*		*per cent*
1	0.5 × 11	(++++)	42.9	(+++)	32.5
15	0.5 × 6	(++)	21.0	(++)	20.0
17	0.5 × 7	(+++)	28.0	(++)	17.0
18	2.0 × 2	(++++)	44.3	(+++)	28.1
19	2.0 × 3	(++++)	37.6	(++)	20.8
22	2.0 × 4	(+++)	27.1	(±)	2.0
23	2.0 × 2	(+++)	31.4	(++)	17.0
			36.0 ± 9.1		19.6 ± 9.6

Acetylsulfanilamide tested in similar concentrations and under the same conditions never produced methemoglobin, thus demonstrating the importance of the free amino group for the reaction.

These preliminary *in vitro* studies are of interest, first, because they help explain the ease with which chickens can form methemoglobin, since these birds normally have a temperature higher than most mammals. Secondly, if sulfanilamide goes to some other compound in the body to form methemoglobin (17) this formation of a new compound may possibly take place *in vitro* also.

THE EFFECT OF METHYLENE BLUE ON EXPERIMENTAL CYANOSES

Since methylene blue has been reported to be successful in combatting methemoglobinemia which follows sulfanilamide therapy in man

(6, 7), it seemed worth while to test the effectiveness of this dye against the chicken and mouse cyanoses.

Cyanosis was produced in a group of chickens by the administration of sulfanilamide, and the percentage of inactive iron was determined by the methods already described. Methylene blue in doses of 5 to 10 mgms. per kilogram was injected intravenously. One to one and a half hours later the percentage of inactive iron was determined again. Table 4 gives a summary of results obtained from experiments on 7 chickens. It can be seen that methylene blue exerted a definitely beneficial effect in many of the birds. Grossly the bloods, following the injection of the dye, were less dark in color, but the results were only rarely as striking as those reported by Hartmann and his coworkers in human cases. It must be borne in mind however that the level of sulfanilamide in the blood of these birds was much higher than that seen in human cases, hence one would expect methylene blue to be working against greater oxidative forces in the birds than in man.

In contrast to the effect in chickens, cyanosis in mice was practically uneffected by methylene blue. Because of the size of the animal it was not practical to do blood studies both before and after the administration of methylene blue in the same mouse. Instead, 24 mice in which cyanosis was induced by feeding a standard diet of sulfanilamide, were divided into 2 groups by random selection. One group was injected with 0.9 per cent saline and the other group was injected with 10 mgms. methylene blue per kilogram intraperitoneally. One hour later bloods were studied according to the methods already described. The average of determinations of inactive iron in the mice receiving saline injections was 15.5 per cent, while the average per cent inactive iron in the group receiving methylene blue was 13.2 per cent, and there was no difference in the color of the bloods. While the number of mice included in these experiments is small, the results are consistent with the findings of sulfhemoglobin in this species under these conditions.

DISCUSSION

The successful production of sulfanilamide cyanosis in chickens, pigeons and mice can probably largely be attributed to the main-

tenance of a high blood level for a considerable period of time. In the case of chickens the rôle of the kidney must be of great importance. In this species glomerular filtration is so low that the drug is excreted very slowly (15). A number of the birds studied showed high blood levels of sulfanilamide 2 to 3 weeks after a few days treatment with the drug. It is quite possible that delayed excretion may to a large extent explain the so-called delayed toxicity reported by some investigators (19).

Because of the feeding habits of mice a constant blood level is maintained by continuous absorption rather than by delayed excretion. It is possible to produce cyanosis in this species by repeated, daily, large doses of sulfanilamide but the results are irregular and likely to be associated with systemic symptoms. The explanation for the formation of a sulfhemoglobin-like pigment must await further work. It is probably not a question of a different type of hemoglobin because the incubation of mouse blood with sulfanilamide *in vitro* results in the formation of methemoglobin just as in the case of chicken and human blood. It seems more likely that the type of diet may furnish the answer.

Most pharmacological studies in animals are of greatest interest when considered in terms of how the results may apply to man. In the case of experimental sulfanilamide cyanosis the transfer of results from one animal to another may be extremely hazardous. The results presented, although lending support to the view that non-functional iron pigment may be of major importance in the etiology of cyanosis in man, do not necessarily rule out the possibility that some other factor may also be operating. From the fact that the pigment formed in chickens and mice was shown to be different, it would not be impossible that still a third factor might be of importance in another species. It would seem that the question of human cyanosis can only be answered by further work done according to the criteria outlined in the first part of this paper.

SUMMARY

1. Methods of inducing sulfanilamide cyanosis in chickens and mice are described.

2. In chickens the cyanosis is explained by the formation of methe-

moglobin. In mice the presence of sulfhemoglobin accounts for the cyanosis.

3. Under the conditions described, sulfanilamide will convert hemoglobin into methemoglobin *in vitro*.

4. Methylene blue exerts a beneficial effect on the sulfanilamide cyanosis in chickens, but not on the mouse cyanosis.

I am indebted to Dr. E. K. Marshall, Jr. for advice and guidance in this problem and wish to thank Miss Dorothea Babbitt for technical assistance with some of the analyses.

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THE COINCIDENCE OF DIABETES MELLITUS AND ADDISON'S DISEASE

Effect of Cortical Extract on Glycemia and Glycosuria

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The coincidence of diabetes mellitus and Addison's disease in the same patient seems to be excessively rare. No proved instance of adrenal disease occurred in Joslin's large series of diabetics (1) and it was not until 1927 that Arnett (2) reported an unequivocal case. Arnett reviews the literature and points out that in all of the five previously reported cases of alleged diabetes and Addison's disease the diagnosis was based on inadequate or questionable data. Since 1927 we have been able to find only three more definite instances (3), although Allan (4) in a brief note refers to two cases seen at the Mayo Clinic among 115 patients with Addison's disease and over 3000 diabetics.

Even more rare is the demonstration of decreased need of insulin as Addison's disease develops in a patient previously diabetic. Unverricht (3) reports a poorly documented case of diabetes and tuberculosis in which the insulin requirement is said to have decreased from 40 units to 10 units as the patient developed Addison's disease. Allan (4), on the contrary, states that suprarenal insufficiency did not have a favorable effect on diabetes and in Levy-Simpson's case (3) glycosuria came on during the course of Addison's disease although insulin gave severe hypoglycemic reactions.

The case herewith reported seems then to be unique in the literature.

CASE REPORT

Part I. The original diagnosis of uncomplicated diabetes mellitus

F. L. (A79862), a 30-year old American electrician, entered the hospital April 18, 1938, with complaint of weakness and loss of weight

for three weeks. One sister died of diabetes at the age of 16 and also a niece at the age of three years. The patient's habits were not unusual and there was no story of trauma or severe infection.

In March 1938, out of a clear sky, came increased thirst and polyuria. He lost about 18 pounds in two months and became quite weak and short of breath on moderate effort.

On examination he was a thin, intelligent, alert young man. General physical examination was not remarkable. The eyes were normal, heart and lungs clear, and abdomen negative. There was no disturbance of the leg vessels. The breath had a slightly fruity odor (acidosis) and the respirations were twenty to the minute. Weight 49 kg. B.P. 120/75. There was a slight elevation of temperature and pulse rate. *No mention of dark skin color is made in notes by numerous examiners.*

Laboratory examinations: R.B.C. 5.2 M. Hbg. 17.5 gm. W.B.C. 12,000. Sed. rate 22 mm. in one hour. Hematocrit 47 per cent. Urine clear; sp.gr. 1.029, heavy reduction of sugar; tests for acetone and diacetic acid strongly positive. Blood CO_2 combining power 59 vols per cent. Cholesterol 178 mg. per 100 cc. X-rays of chest showed normal heart, aorta, and lung fields.

Course: The blood sugar on entry was 560 mg. per 100 cc. He was placed on a diet of approximately 2000 cals. (C.150, P.80, F.120) with insulin and soon became sugar free. April 22, blood sugar 183; April 26, 118. He was discharged April 28th on the above diet with insulin 56 units (Protamine 32 U., old insulin 24 U.).

Observation during the hospital stay showed straight forward diabetes. There was no suggestion of disease of pituitary, adrenals, or thyroid. He gained weight from 49 to 52 kg., the temperature became normal, and he felt perfectly well.

Part II. The development of Addison's disease with lessening of insulin requirement

After leaving the hospital the patient was followed in the diabetic clinic. During May and June 1938 it was possible to reduce his insulin to approximately 40 U. (protamine) daily, and to maintain him sugar free on a diet of 1800–2000 cals. During July and August, however, attempts to reduce the insulin further were associated with

marked glycosuria. After his visit to the clinic on August 16, 1938, he felt so well that he did not return and while he states that he continued to eat approximately the same diet, he gave up accurate control. During the fall and winter, until March 1939, he felt perfectly well and was at work, but in December 1938 he reduced his insulin because of a reaction. A number of subsequent hypoglycemic reactions occurred and the insulin was further lessened by the patient to 4 U. of old insulin once or twice daily. No sugar appeared in the urine as tested at home by himself and his wife. Despite the lower insulin requirement he states he continued to eat about the same quantity of food and lost no weight until later on.

In the latter part of March 1939 a new train of symptoms developed in spite of the fact that he was said to be sugar free. He became extremely weak, in fact exhausted, and had to quit his job. With this came shortness of breath on slight effort. His appetite went away completely, but he began to note a craving for salt. He became constipated and later had attacks of nausea. At about this time a change of skin color was observed by both the patient and his wife who described it as a yellow brown. He was very sensitive to cold. There was moderate weight loss. About two weeks before re-entry to hospital on May 11, 1939, he had what was probably a hypoglycemic attack consisting of a spell of confusion and agitation during which he threw objects about and threatened his wife. Later, after taking sugar, he had no recollection of this spell. Finally he felt so badly that he consulted Dr. L. R. French, who sent him into the hospital.

Part III. Evidence that the patient had Addison's disease

Hospital re-entry May 11, 1939. He presented a tired, somewhat apathetic appearance. The skin was of a deep gray-brown dingy tint. This color affected the body as well as the exposed parts. There was some accentuation at the body folds, and over the upper back were many almost black freckle-like spots. No abnormal buccal pigmentation was seen. Muscular strength was distinctly weak and he complained of exhaustion. Nutrition was fairly well preserved and he weighed 55.5 kg. in contrast to 49 kg. on the first entry. The lungs were perfectly clear and there was no evidence of tuberculosis

elsewhere. The heart was clear. Pulse 70–90. Abdomen negative; liver and spleen not felt. Blood pressure 85/60.

Laboratory tests: Urine, on entry (4:30 p.m.) clear; sp.gr. 1.024; a trace of sugar; no acetone. Blood CO_2 combining power 55.5 vols per cent. Cholesterol 217. Blood sugar (May 12—fasting in a.m.) 105 mg. per 100 cc. Blood: R.B.C. 4.6 M.; Hbg. 87 per cent (Shali); W.B.C. 9,200. Hematocrit 45 per cent. Sed. rate 21 mm. per 1 hour. Blood chlorides (as NaCl, whole blood) 385 mg. per 100 cc. Blood urea (May 15) 57 mg. per 100 cc. Wassermann negative. Stool normal. Plasma proteins 6.2 gm. per cent. Icteric index 4 U. Van den Bergh 0.8 U. Tuberculin 1–10,000 i.d. = + in 24 hours. B.M.R. −13 per cent. Visual fields normal. X-rays of abdomen, chest and sella turcica normal. No areas of calcification were seen in the adrenal regions. No hemosiderin granules were found in the urinary sediment. Biopsy of skin showed only melanin and no iron containing pigment (Dr. Cox).

We first thought of hemochromatosis as an explanation of his acute symptoms and pigmentation. But the fact that the diabetes was better rather than worse, the absence of palpable liver and spleen, and finally the skin biopsy seemed to rule out this possibility. The diagnosis of Addison's disease on the other hand was established by (a) typical pigmentation, (b) great asthenia, (c) anorexia and nausea, (d) very low blood pressure, (e) salt hunger, (f) very low blood chloride, (g) elevated blood urea, (h) hemoconcentration (see below), and (i) relief of symptoms by cortical extract and salt (see below).

Part IV. Effect on glycosuria and hyperglycemia of injections of adrenal cortical extract

It has been established experimentally that adrenal cortical insufficiency may result in hypoglycemia. In addition there is said to be a diminution in the glycogen content of liver and muscles and a delay in the mobilization of glycogen from glucose or lactic acid (5). These observations are in harmony with clinical experience of spontaneous hypoglycemia or at least increased insulin sensitivity in patients with Addison's disease. Furthermore, it is known that the glycosuria of experimental pancreatectomy may be ameliorated by excision of the adrenals (cortex), and that potent cortical extracts

not only maintain normal carbohydrate levels in adrenalectomized animals but increase the blood sugar in animals deprived of both pancreas and adrenals (5). The effect of adrenal cortical hormone

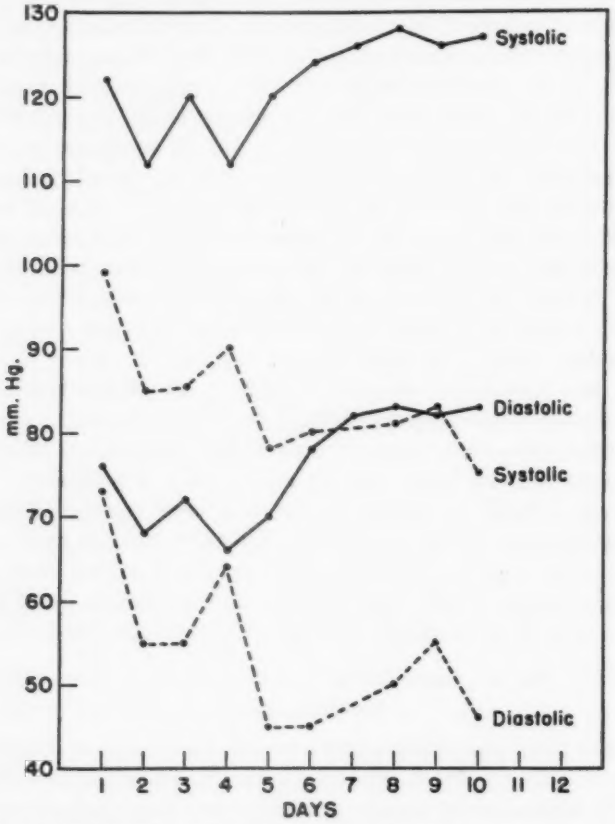

FIG. 1. Blood pressure during first hospital admission •———•, and during second hospital admission •– – – –•.

was therefore studied in our patient to see whether similar responses would be obtained.

On entry the patient was quite ill. His extremities were very cold and he complained bitterly of nausea. He fainted on getting off the examining table to go back to bed, and two days later had a short

attack of syncope and coldness which passed off spontaneously. He was placed on a diet of 1600 cal. (C.130, P.70, F.90 gm.) containing not over 0.5 gm. of salt; and NaCl 20 gm. was given daily in the form of "Enseals." Because of anorexia and nausea it was impossible to make him eat the full diet at first, but it was obvious that very little insulin was necessary. On the high salt intake he promptly improved and, while he still felt somewhat weak and the appetite remained poor, within a week after entry the blood chlorides had

TABLE I

Effect of cortical extract on glycosuria and glycemia

DATE, 1939	CALORIES CONSUMED PER DAY	UNITS INSULIN PER DAY	BLOOD SUGAR FASTING IN A.M.	GRAMS SUGAR PER 24 HOURS URINE	BLOOD CHLORIDE AS NaCl (WHOLE BLOOD)	REMARKS
			mg. per 100 cc.		*mg. per 100 cc.*	
			105 (May 12)		385 (May 12)	Blood urea 57 (May 15) (Diet: P. 70, C. 130, F. 100)
					524 (May 18)	Blood urea 45 (May 20)
May 25	1527	10	86	1.0	541	Blood urea 39
May 26	1215	10		0		
May 27	1429	10		0		Eschatin 20 cc.
May 28	1345	10		0		Eschatin 20 cc.
May 29	1641	10	109	0	549	Eschatin 30 cc.
May 30	1622	10	244	15.5		Eschatin 30 cc.
May 31	1601	10		2.9		
June 1	1535	10		2.9		
June 2		10	190	0		

risen from 385 to 524 mg. per 100 cc. (as NaCl in whole blood), blood urea dropped from 57 to 45 and later to 39 mg. per 100 cc. Hemoconcentration lessened as indicated by a fall in hematocrit from 45 per cent tc 29 per cent, and a drop in hemoglobin from 87 per cent to 58 per cent (Sahli). It was calculated that there was an increase in blood volume of nearly a liter. Blood pressure remained essentially unchanged (see chart).

By May 25th he was fairly well stabilized with 6 units of insulin before breakfast and 4 units before supper. It was still difficult to

make him eat the full 1600 cal. diet, but on a consumption of from 1300 to 1600 cal. he remained sugar free. Events during the next few days are best shown in Table 1. *Eschatin* was given intramuscularly, 10 cc. twice daily for two days and 10 cc. thrice daily for two days. It appears that there was an increased output of sugar and the blood sugar (fasting in a.m.) rose on May 30th from previous readings of approximately 100 to nearly 250 mg. per 100 cc. As soon as the eschatin was discontinued the blood sugar began to fall and the output of sugar in the urine dropped to the levels obtaining before eschatin was given. There was extraordinary subjective improvement beginning on the third eschatin day and continuing up to his discharge from the hospital on June 2d.

These observations suggested that cortical extract increased glycemia and glycosuria in our patient. The results did not seem to us, however, entirely conclusive and it was thought desirable to try the effect of the synthetic hormone, desoxy-corticosterone acetate. When a supply of this substance was available through the courtesy of Ciba Pharmaceutical Products, the patient was asked to return to the hospital for further study.

Part V. The effect of desoxy-corticosterone acetate in a patient with diabetes mellitus and Addison's disease

On July 3d, 1939, the patient returned. He had not been careful as to diet or insulin and he seemed dull and lethargic. The physical findings were essentially unchanged. B.P. 80/50. Blood urea 27 mg. per 100 cc. Blood NaCl (whole blood) 503 mg. per 100 cc. He was placed on the same regimen as before: C.130, P.70, F.90 = 1600 cals. with insulin 6–0–4, and 10 gm. of extra salt daily. On the morning after entry, however, the blood sugar was found to be 310 mg. per 100 cc. and on this day he was given insulin 6–10–12. At 11 o'clock (p.m.) he was found in severe insulin shock from which he was revived by glucose intravenously and adrenalin. During subsequent days it became clear that he was excessively sensitive to insulin but promptly became hyperglycemic if his small dose of insulin was lowered. By July 8th he seemed fairly well stabilized on a diet of C.125, P.60, F.75 = 1400 cals. with insulin 6–0–4. On this regimen he was completely sugar free and had no clinical hypo-

glycemia although the fasting blood sugar ranged from 50 to 70 mg. per 100 cc. Desoxy-corticosterone acetate (in oil, injected subcu-

TABLE II

Effect of desoxy-corticosterone acetate on glycosuria and glycemia

DATE	CALORIES CONSUMED PER DAY	BLOOD SUGAR FASTING IN A.M.	GRAMS SUGAR PER 24-HOURS URINE	INSULIN	CORTICAL HORMONE
		mg. per 100 cc.			
July 7	1396	74	0	6-0-4	None
July 8	1435		0	6-0-4	Desoxy-corticosterone acetate 5 mgm.
July 9	1387		0	6-0-4	Desoxy-corticosterone acetate 10 mgm.
July 10	1375		0	6-0-4	Desoxy-corticosterone acetate 15 mgm.
July 11	1377	51	0*	6-0-4	Desoxy-corticosterone acetate 25 mgm.
July 12	1456	50	0*	6-0-0	
July 13	1416		21	0-0-0	
July 14	1419	188	20	0-0-8	
July 15	1475	76	0	4-0-0	

* Insulin reaction.

TABLE III

Effect of natural and synthetic cortical hormones

DATE	CALORIES	BLOOD SUGAR	URINE SUGAR	INSULIN	CORTICAL HORMONE
		mg. per 100 cc.			
July 24	1423	66	0	4-0-2	—
July 25	1386	106	0	4-0-2	—
July 26	1445	64	0	4-0-2	—
July 27	1329	68	0	4-0-2	Eschatin 30 cc.
July 28	1424	80	0	4-0-2	Eschatin 30 cc.
July 29	1438	75	0	4-0-2	Eschatin 30 cc.
July 30	1427	107	0	4-0-2	Eschatin 40 cc.
July 31	1380	105	0	4-0-2	—
August 1	1324	148	0	4-0-2	—
August 2	1437	72	0	4-0-2	Desoxy-corticosterone acetate 15 mgm.
August 3	1391	82	0	4-0-2	Desoxy-corticosterone acetate 20 mgm.
August 4	1436	59	0	4-0-2	Desoxy-corticosterone acetate 25 mgm.
August 5		56	0	4-0-2	Desoxy-corticosterone acetate 25 mgm.

taneously) was started on July 8th with the results shown in Table II. It may be seen that not only was there no lessening of sugar tolerance on the synthetic hormone but the blood sugar actually fell. The

last few days of the chart show the extreme difficulty of stabilizing this patient.

Beginning July 18th ordinary insulin was changed to crystalline insulin. On the 1400 calory diet with crystalline insulin 4–0–2 he was sugar free with fasting blood sugar varying from 60 to 105 mg. per 100 cc. Eschatin was tried once more as shown in Table III. While the effect was not so striking, it clearly resembles that seen in Table I; after several days of eschatin there was a definite tendency for the blood sugar to be higher and this continued for two days after the extract was stopped. Administration of desoxy-corticosterone acetate on the other hand had no effect and as in the previous trial (see Table II) was associated with falling blood sugar. During the entire hospital stay he felt fairly well and there was no notable difference in his clinical condition with eschatin as against the synthetic hormone.

DISCUSSION

Desoxy-corticosterone acetate given in two experimental periods of several days each quite clearly did not produce hyperglycemia or glycosuria in a patient with Addison's disease and diabetes mellitus under control with small doses of insulin. The synthetic hormone failed therefore to produce the exacerbation of the diabetic state which has been described in depancreatized and adrenalectomized animals treated with cortical extracts. A non-synthetic cortical extract—eschatin—appears on the other hand to have raised blood sugar and even produced glycosuria in our patient. Unfortunately in the first trial (Table I) the experiment may not be absolutely binding inasmuch as the patient's caloric intake was slightly higher on the days when the glycosuria occurred. On May 30th for example he ate 130 gms. of carbohydrate as against 112 on May 28th. However during a long control period there were many days on which 120–130 gm. of carbohydrate were ingested without glycosuria or hyperglycemia. Again during the second trial of eschatin (Table III) the blood sugar seems clearly to have risen; but the fluctuations were already wide during the control period as witnessed by the figures obtained on July 24, 25 and 26. We may sum it up by saying that the results with eschatin while suggestive are not conclusive,

but those with the synthetic hormone are unequivocally negative as regards exacerbation of the diabetes.

SUMMARY

1. A patient with uncomplicated diabetes subsequently developed frank Addison's disease. The exact nature of the adrenal lesion was not established, but there was no proof of tuberculosis or of tumor.

2. As Addison's disease developed less insulin was necessary to maintain the patient sugar free; his requirement dropped from 40 units to 4 to 6 units and there were hypoglycemic reactions.

3. Injections of synthetic adrenal cortical hormone—desoxycorticosterone acetate—on two occasions did not produce an increase of glycosuria or glycemia. On the contrary, the blood sugar seemed to be lowered. Eschatin administration, on the other hand, over periods of three or four days in large doses was followed by higher blood sugars and in the first trial by increased glycosuria. The difference in the two preparations seemed quite definite.

4. Insofar as eschatin produced glycemia, these observations parallel the effects of injection of cortical extract in depancreatized and adrenalectomized animals. This appears to be the first instance in which an attempt has been made to reproduce these findings in man.

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FURTHER STUDIES ON THE EFFECT OF SULFANILAMIDE ON EXPERIMENTAL TUBERCULOSIS

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In 1938, Rich and Follis (1) reported experiments in which it was shown that the development of tuberculosis in the guinea pig was markedly inhibited by sulfanilamide administered daily by mouth from the beginning of the experiments. It was shown in that study that the dosage of the drug was an essential factor in the results obtained, and that only equivocal or negative results could be expected from an inadequate dosage. Two hundred and fifty milligrams of sulfanilamide per kilogram of body weight, given daily in a single dose throughout the entire course of the experiment, had no effect upon the development of the disease; 500 mg. per kilo. had what was regarded as a significant but only moderate effect; while 1250 mg. per kilo. exerted a very striking inhibitory effect. In those experiments it was shown that the guinea pig acetylates the drug to a much greater degree than does the human being and that, consequently, much larger doses are required in the guinea pig than in the human being in order to attain an appreciable concentration of the free drug in the blood.

Following the publication of our experiments there appeared a number of studies of the matter by different investigators (2, 3, 4, 5, 6, 7). Most of these investigators have confirmed our observation that sulfanilamide inhibits the development of experimental tuberculosis, but there have been differences in the degree of inhibition observed by different investigators, and some obtained no inhibitory effect at all in their experiments. Examination of these various reports shows plainly that those who have observed the inhibitory effect have used doses of sulfanilamide at least approximating that which we stated to be necessary for that result, and they have also adhered to the requirements of administering the daily amount in broken doses in order to maintain an effective concentration in the blood, and con-

tinuing the treatment throughout the experiment; while those who have concluded that the drug does not affect the progress of the infection have neglected these important factors. For example, Kolmer, Raiziss and Rule (5) reported that sulfanilamide exerted no effect upon experimental tuberculosis in the guinea pig. Four animals were treated daily with 200 mg. per kilo., four with 300 mg. per kilo., and four with 500 mg. per kilo. It will be recalled that in our experiments 500 mg. per kilo. had only a moderate effect, and that 1250 mg. per kilo. was necessary for a really striking inhibition of the infection; and furthermore, that in our studies the drug was administered throughout the duration of the experiment. In contrast, Kolmer, Raiziss and Rule (5) administered the above inadequate doses for only eight days and then stopped treatment entirely.

Again, Smithburn (2) reported that in his hands sulfanilamide exerted no effect upon experimental tuberculosis in the guinea pig. The dose per kilo. of body weight that he used, however, was less than half of that which we found to be necessary for a pronounced effect upon the infection, and, very important, Smithburn (2) administered the drug in a single daily dose—a procedure which renders it impossible to maintain an effective blood level.

That the success or the failure of different investigators to obtain an inhibitory effect upon the development of experimental tuberculosis is to be correlated with the dosage and the manner and duration of administration of sulfanilamide, may be clearly seen in Table I.

In our previous paper it was shown that in the guinea pig it is much more difficult than in the human being to attain a high level of free sulfanilamide in the blood. This is also true in the rabbit. In both of these animals differences in the degree of acetylation of the drug and, apparently, differences in absorption, excretion or both, make it necessary to administer much larger doses than are necessary in the human being in order to attain and maintain an effective blood concentration of free sulfanilamide. Since the drug is excreted by the kidney and concentrated in the urine, we thought that perhaps additional evidence of its activity might be obtained by studying its effect upon renal tuberculosis. As is well known, the guinea pig practically never develops renal tuberculosis during generalized infection, while the rabbit does so almost always. In view of this marked suscepti-

bility of the rabbit's kidney to tuberculosis, we carried out the present experiments on rabbits. Since the rabbit is natively resistant to the human type of tubercle bacillus and highly susceptible to the bovine type, the latter type was used as the infecting organism. The bacilli were injected intravenously. This route of infection produces a more rapidly progressing generalized tuberculosis than that caused by

TABLE I

Relation of Dosage, Method and Duration of Administration of Sulfanilamide to the Effect upon Experimental Tuberculosis

	TREATMENT BEGUN IN RELATION TO INFECTION	TREATMENT WITH SULFANILAMIDE				DURATION OF EXPERIMENT	EFFECT ON PROGRESS OF INFECTION
		Total daily*	How administered daily	Route of administration	Duration of administration		
		mg. per kilo	*doses*		*days*	*days*	
Rich and Follis (1)	3 days before	1,250	4	Oral	42	42	Inhibition
Ballon and Guernon (6)	5 days after	1,250–985	4	Oral	35	35	Inhibition
Greey et al. (4)	3 days before	860	3	Oral			Inhibition
Greey et al. (7)	3 days before	860	4	Oral	50	50	Inhibition
Buttle and Parish (3)	On day of infection	625	4	Oral	42	42	Some degree of inhibition
Smithburn (2)	On day of infection	570	1	Intra-abdominal	30	49	No effect
Kolmer et al. (5)	On day of infection	500	2	Intra-muscular	8	66	No effect

* The dosage listed here represents that which was administered during the greater part of the experiment. In some cases the dosage was changed for several days.

infection by any other route and thus provides a severe test of the efficacy of a therapeutic agent.

SULFANILAMIDE IN EXPERIMENTAL TUBERCULOSIS IN THE RABBIT

The bovine tubercle bacillus used in these experiments was a virulent strain (Ravanel) obtained through the courtesy of Dr. Max B. Lurie. It was grown on Löwenstein's medium. In testing the effect of sulfanilamide on tuberculous infection in rabbits, four series of animals

were studied. Whenever a treated animal died an untreated control animal was killed to compare the extent of the lesions. Autopsies were performed as promptly as possible, and microscopic sections were made of both lungs, both kidneys, the liver and the spleen of every animal. All of the rabbits averaged about 2000 gm. in weight; all received the same food and were kept in the same environment. The sulfanilamide (Merck) used was in a finely powdered form. Measured doses were deposited in the cheek pouch. For this purpose we used a properly graduated piece of heavy glass tubing with a glass rod for a plunger. A thick disc of cork was fitted into the tube in front of the glass rod. In Series I and III the drug was administered in the dry state. Since some of the powder was lost from the mouth before it became moistened by saliva, in Series II and IV the material was moistened with water immediately before administration. After filling the glass tube with the desired amount of the powdered drug, the tube was connected by a rubber hose to the water faucet and water was gently forced through the powder. The powder was thus converted into a thick paste which could be injected in measured amounts into the cheek pouch and smeared about with the end of the tube.

Series I. This consisted of 20 rabbits, 16 of which (8 treated and 8 controls) lived 15 to 37 days after infection. The animals were infected intravenously with 0.5 cc. of a saline suspension of bovine bacilli that contained 15–20 organisms per oil emersion field. Treatment with sulfanilamide was begun the day of infection. Two hundred milligrams were given at 9 a.m., 200 mg. at 2 p.m., 200 mg. at 6 p.m., and 400 mg. at midnight. The total dose (1000 mg.) amounts to 500 mg. per kilo of body weight each day. This program was carried on for 11 days. On the 12th day the dose was raised to 300, 200, 300 and 400 mg., given at the above hours for the remainder of the experiment. This total dose (1200 mg.) amounts to 600 mg. per kilo. of body weight each day. For obvious reasons, both in this series and in the following ones this precise schedule could not be adhered to each day; however, each treated animal always received four doses of the drug in the amounts stated above, spaced as equally as possible each day. Two of the treated animals died in the 3rd week; 3 in the 4th week; 2 in the 5th week, and the remaining animal was autopsied in the 6th week.

As was noted above, animals in this series died in the 3rd to the 6th week after infection. There was a definite difference, though it was not marked, in the degree of involvement of the lungs of the treated and untreated animals dying in the 3rd week. There was less casea-

tion in the treated rabbits. However, in the other organs no appreciable differences could be found. There was little involvement of the kidneys in either group and the splenic tuberculosis was about the same in both. Those animals coming to autopsy in the 5th and 6th weeks revealed no differences whatsoever in the degree of involvement of the various organs in either group. Involvement of the lungs was marked. Lesions in the kidneys were fairly extensive and no difference in the two groups could be found.

In Table II are shown the concentrations of free and combined sulfanilamide in the blood and urine of 5 animals in this series. The method of Marshall (8) was used in making these determinations. From the observations in this table it will be seen that there was a tendency for the concentration of free sulfanilamide in the blood to be rather low. The urine of the two animals tested likewise had relatively little of the free compound.

Summary. In this series, except for a difference in pulmonary involvement in the treated and untreated animals dying in the 3rd week after infection, no effect whatsoever from then on could be noted in the tuberculous lesions in the lungs, livers, spleens, and kidneys of the untreated control animals and those animals to which sulfanilamide had been administered daily in doses of 500 to 600 mg. per kilo. of body weight.

Series II. In this group 22 animals were infected, 14 of which lived 39 to 79 days. Six of these were treated and 8 served as controls. In this series the intravenous infecting dose was 0.5 cc. of a suspension containing approximately 10 organisms per oil emersion field. The next day, 0.01 cc. of a suspension containing the same number of organisms was injected into the anterior chamber of one eye. Treatment was begun the day of the intravenous injection and consisted of 400, 200, 200, and 400 mg. sulfanilamide administered at 9 a.m., 2 p.m., 6 p.m., and midnight. This total dose (1200 mg.) amounts to 600 mg. per kilo. of body weight each day. One of the treated animals came to autopsy in the 6th week, 2 in the 8th week, and one each in the 10th, 11th, and 12th weeks.

As noted above, animals in this group lived from the 6th into the 12th week. As far as the pulmonary lesions were concerned, no appreciable difference could be detected between the treated and untreated animals coming to autopsy at the same time. Nor was the involvement of the spleens and livers of the two groups of sufficient

difference to comment upon. However, the difference in the degree of involvement of the kidneys in the treated and untreated rabbits was striking (Fig. 1). Little renal tuberculosis in either group was seen

TABLE II

Series I

Animal number	Length of life		Concentrations of sulfanilamide in mg. per 100 cc. at intervals during experiment											
			Blood								Urine			
			1/14/38*	1/20/38	1/27/38	2/2/38	2/4/38	2/10/38	2/16/38	Average	2/2*	2/4	2/10	2/16
	days													
2	37	F	1.9	1.8	1.5	1.5	2.8	1.9	2.6	1.5	100	220	218	75
		C		3.9	3.4	2.8	6.5	5.0	7.4	4.8	900	1580	1450	750
		T		5.7	4.9	4.3	9.3	6.9	10.0	6.3	1000	1800	1668	825
4	31	F	2.0	1.4	1.8	5.0	2.9	2.9	Dead	2.7	180	230	70	
		C		2.8	4.0	4.0	5.4	9.5	"	5.0	140	1620	1180	
		T		4.2	5.8	9.0	8.3	12.4	"	7.8	820	1950	1250	
10	22	F	3.7	1.9	5.5	Dead				3.7				
		C		2.7	4.5	"				3.6				
		T		4.6	10.0	"				7.3				
12	23	F	3.0	1.3	4.3	6.5	Dead			3.8				
		C		2.3	5.2	10.1	"			5.9				
		T		4.6	9.5	16.6	"			9.7				
6	22	F	10.0	4.2	10.0	Dead				8.1				
		C		5.5	8.2	"				6.8				
		T		9.7	18.2	"				14.9				

F, free sulfanilamide. Date of infection: 1/11/38.

C, conjugated sulfanilamide.

T, total of free and conjugated sulfanilamide.

* Dates: 1/14/38, blood obtained 4 hours after administration of 200 mg.; 1/20/38, blood obtained 4 hours after administration of 200 mg.; 1/27/38, blood obtained 4 hours after administration of 300 mg.; 2/2/38, to 2/16/38, blood obtain 9 hours after administration of 400 mg.

Urine was obtained by catheterization in morning, 9 hours after administration of 400 mg.

until the 8th week. From this time on there began to be rather marked involvement of the kidneys of the control group but in only one treated animal was the renal tuberculosis as extensive as in the

control coming to autopsy at the same time. No appreciable difference was noted in the ocular involvement in the treated and untreated animals.

Since in this series only a few determinations were made of the concentration of sulfanilamide in the blood and urine of the animals, the data obtained are not sufficient to warrant recording.

Summary. In this group, no difference in the tuberculous involvement of the lungs, livers, and spleens could be noted. However, involvement of the kidneys of the untreated controls was more extensive from the 8th week on than in the animals treated with sulfanilamide administered daily in doses of 600 mg. per kilo. of body weight for 79 days.

Series III. This group consisted of 14 animals of which 13 (6 treated and 7 controls) lived 12 to 24 days after infection. All the animals were infected intravenously with 0.1 mg. of the virulent bovine organisms suspended in 0.5 cc. of saline. Sulfanilamide therapy was begun the day of infection. The treated animals received 300 mg. at 9 a.m., 300 mg. at 2 p.m., 300 mg. at 7 p.m., and 600 mg. at midnight. The total amount given each day (1500 mg.) amounted to 750 mg. per kilo. of body weight. One treated animal was killed in the 2nd week; 4 died in the 3rd week, and the remaining one died in the 4th week.

At the 12th day after infection there were macroscopic lesions in the lungs of the control while very little was seen in the treated animal autopsied at this time. Microscopically, too, the pulmonary lesions in the treated animal were smaller than in the control. The liver and spleen of the control revealed a few tuberculous foci while those of the treated rabbit showed nothing. The kidneys of neither animal were involved. In the ensuing 12 days of the experiment the pulmonary lesions in the controls were always more extensive than those in the animals treated with sulfanilamide and coming to autopsy at the same time (Figs. 2 and 3). Especially striking at the end of the 3rd week were the differences found in the kidneys of the two groups. Numerous macroscopic foci studded the cortices of the kidneys of the untreated animals while only a few minute foci could be detected in the treated rabbits (Figs. 2 and 3). The spleens of the untreated group were larger and microscopically tended to be more involved than in the treated animals. Little difference was noted in the hepatic involvement.

A few determinations on the concentration of free and combined

sulfanilamide in the blood of three animals and in the urine of one animal, were kindly performed by Dr. E. K. Marshall, Jr. The results are shown in Table III. These determinations served to indicate the marked variation in the blood levels of three animals receiving approximately the same amount of sulfanilamide by mouth.

Summary. When 750 mg. of sulfanilamide per kilo. of body weight was administered in divided daily doses there was a definite difference in involvement of the kidneys of the treated and control animals. In the lungs, too, the tuberculous lesions were less marked in the former group.

TABLE III

Series III

ANIMAL NUMBER	LENGTH OF LIFE		CONCENTRATIONS OF SULFANILAMIDE IN MG. PER 100 CC. AT INTERVALS DURING EXPERIMENT				
			Blood				Urine
			11/19/37	11/20/37	12/3/37	Average	
	days						
65	24	F	0	1.5	0.08	0.5	
		C	1.5	3.6	0.3	1.8	
		T	1.5	5.1	0.38	2.3	
61	16	F	1.5	2.4	Died	2.0	
		C	4.0	0	"	2.0	
		T	5.5	2.4	"	4.0	
63	17	F	20.0	14.2	15.2	16.4	961
		C	0	0	0	0	289
		T	20.0	14.2	15.2	16.4	1250

F, free sulfanilamide.

C, conjugated sulfanilamide.

T, total of free and conjugated sulfanilamide.

Date of infection: 11/16/37. 11/19/37, blood obtained 9 hours after administration of 600 mg.; 11/20/37, blood obtained 4 hours after administration of 300 mg.; 12/3/37, blood obtained 9 hours after administration of 600 mg.

Urine obtained 9 hours after administration of 600 mg.

Series IV. This group consisted of 28 rabbits, all of which were infected intravenously with 0.25 cc. of a suspension containing approximately 16 tubercle bacilli per oil emersion field. Eighteen animals were treated with sulfanilamide and 11 of these survived 24 to 72 days, at which time the last survivor was killed. In Table III the survival time for each animal is given. The dose of drug ad-

ministered was 300 mg. at 9 a.m., 300 mg. at 2 p.m., 300 mg. at 6 p.m., and 600 mg. at midnight. The total dose (1500 mg.) amounts to 750 mg. per kilo. of body weight each day. The length of life of each animal is shown in Table IV. Ten animals served as controls of which 9 lived 24 to 72 days.

From the beginning of the 4th week (24 days) to the early part of the 11th week (72 days), the pulmonary lesions in the animals treated with sulfanilamide were with one exception (No. 516) strikingly less extensive than those of the controls coming to autopsy at the same time (Figs. 4–9). In 6 animals treated with sulfanilamide and living 33 to 72 days there were, with that one exception (No. 516), relatively few scattered tubercles in the lungs, while the controls showed extensive caseous lesions. In the kidneys the differences in involvement were even more striking (Figs. 4–9). The controls exhibited extensive renal involvement, the kidneys being studded with tubercles. In marked contrast only a few small scattered lesions could be found in the kidneys of the animals treated with sulfanilamide. The differences in the lesions in the livers and spleens of the two groups were less marked, but in general the involvement in the sulfanilamide-treated animals tended to be less than that in the other group.

Fairly adequate studies were made of the concentrations of sulfanilamide in the blood of the rabbits in this series, and the results are shown in Table IV.

Summary. When sulfanilamide was administered in daily divided doses of 750 mg. per kilo. of body weight, there was much less involvement of the lungs and kidneys of the animals receiving the drug than in the untreated control animals. Like differences, though not so marked, were also noted in the livers and spleens of the two groups.

Differences in the manner in which different rabbits handle a given dose of sulfanilamide. From a study of Table IV a number of interesting points emerge. In the first place, there are marked differences in the ability of individual rabbits to acetylate the drug. Another interesting fact is that while there were rather marked fluctuations in the concentration of the drug in the samples of blood taken on different occasions, nevertheless, there was in most animals a distinct tendency to maintain either a high or a low concentration of sulfanilamide in the blood. It appears that individual animals differ in their inherent ability to handle the drug. Such individual differences in

TABLE IV

Series IV

Animal number	Length of life		Concentrations of sulfanilamide in mg. per 100 cc. at intervals during experiment										
			Blood									Urine	
			10/20/38	10/27/38	11/3/38	11/10/38	11/17/38	11/29/38	12/6/38	12/17/38	Average		
	days												
504	25	F	0.9	0	0	Dead					0.3	131	
		C	10.5	5.3	14.6	"					10.1	743	
		T	11.4	5.3	14.6	"					10.4	874	
503	33	F	5.6	0	1.8	4.0	5.5	Dead			3.4		
		C	7.0	3.2	1.5	5.1	6.1	"			4.6		
		T	12.6	3.2	3.3	9.1	11.6	"			8.0		
517	27	F	6.7	2.4	2.1	5.4	Dead				4.2	1250	
		C	16.3	5.7	7.5	10.5	"				10.0	1460	
		T	23.0	8.1	9.6	15.9	"				14.2	2710	
515	26	F	1.3	2.1	4.5	12.1	"				5.0	136	
		C	4.2	3.6		7.6	"				5.1	445	
		T	5.5	5.7		19.7	"				10.1	581	
506	26	F	0.8	0	2.7	23.8	"				6.8	400	
		C	5.7	3.6	11.6		"				7.0	578	
		T	6.5	3.6	14.3		"				13.8	978	
516	53	F	14.5	4.3	9.3	15.3	20.9	2.1	4.1	Dead	10.7	345	
		C	10.5	6.2	4.9	3.1	17.5	2.6	6.3	"	7.3	845	
		T	25.0	10.5	14.2	18.4	38.4	4.7	10.4	"	18.0	1190	
511	72	F	28.6	12.9	16.7	11.8	14.3	10.0	16.0	4.1	14.3	910	
		C	13.4	5.3	7.1	10.3	8.8	4.3	3.7	2.9	7.0	1090	
		T	42.0	18.2	23.8	22.1	23.1	14.3	19.7	7.0	21.3	2000	
505	38	F	24.2	12.6	22.3	16.5	11.8	Dead			17.5	1120	955
		C	9.2	1.9	0	0	3.4	"			2.9	230	1110
		T	33.4	14.5	22.3	16.5	15.2	"			20.4	1400	2065
512	55	F	20.0	21.8	33.6	20.3	16.5	8.3	12.0	Dead	19.2	1000	
		C	11.2	0.6	3.1	14.5	2.4	0	7.7	"	5.6	180	
		T	31.2	22.4	36.7	36.8	18.9	8.3	19.7	"	24.8	1180	
509	35	F	15.9	14.7	33.9	24.8	16.2	Dead			21.1	3580	
		C	0.6	0.1	4.7	8.8	1.4	"			3.1	430	
		T	16.5	14.8	38.6	33.6	17.6	"			24.2	4010	
529	24	F	25.0	20.4	23.0	Dead					22.8	1250	
		C	10.8	0.2	0.6	"					3.9	195	
		T	35.8	20.6	23.6	"					26.7	1445	

From 10/20/38 to 12/6/38 blood was obtained 8 hours after feeding 600 mg. of sulfanilamide. On 12/17/38 blood was obtained 9 hours after feeding 600 mg. of sulfanilamide.

F, free sulfanilamide. C, conjugated sulfanilamide. T, total of free and conjugated sulfanilamide.

Date of infection: 10/15/38.

Urines were obtained on various dates.

rabbits have also been noted by Engelfried (9). Whether these individual variations are due to differences in absorption or in excretion, or in both, we are unable to say at present. Such differences must play an important rôle in determining the therapeutic effect of a given dose of sulfanilamide upon different rabbits. That such is the case is indicated in the present experiments. By and large, there was definite correlation between the amount of the tuberculosis at autopsy and the concentration of the drug in the blood of the animals during the experiment. It is thus obvious that this factor of individual variation is an important one to be considered in evaluating the therapeutic effect of this drug in experiments carried out on rabbits and probably on other animals.

It will be noted in Table IV that the concentration of free sulfanilamide in the urine reached extraordinarily high levels; as much as 3580 mg. of the drug per 100 cc. of urine was found in one instance. That such concentrations can exist is due, of course, largely to the absorption of water by the renal tubules (14). It is probable that the striking differences in the renal involvement of the treated and untreated animals may be related not only to the concentration of sulfanilamide in the blood but also to the large amounts passing through the renal tissue in the urine, for, if a lesion occurs, diffusion of the drug from ruptured tubules may take place.

SULFAPYRIDINE IN EXPERIMENTAL TUBERCULOSIS IN THE RABBIT

In order to test the effectiveness of sulfapyridine[1] upon experimental tuberculous infection in the rabbit, 10 animals were infected with the same dose and at the same time that the animals in Series IV were inoculated. These animals were treated with daily doses of sulfapyridine consisting of 300 mg. at 9 a.m., 300 mg. at 2 p.m., 300 mg. at 7 p.m., and 600 mg. at midnight. This amounts to 750 mg. per kilo. of body weight. Eight of these animals survived 24 to 72 days. The same controls were used for these animals as were used for the sulfanilamide treated animals of Series IV. The length of life in days after infection is shown in Table V.

[1] Furnished through the courtesy of Dr. D. F. Robertson of Merck and Co., Inc., Rahway, N. J.

TABLE V

ANIMAL NUMBER	LENGTH OF LIFE		CONCENTRATIONS OF SULFAPYRIDINE IN MG. PER 100 CC. AT INTERVALS DURING EXPERIMENT										
			Blood									Urine	
			10/20/38	10/27/38	11/3/38	11/10/38	11/17/38	11/29/38	12/6/38	12/17/38	Average		
	days												
521	55	F	f.tr.	f.tr.	v.f.tr.	v.f.tr.	f.tr.	0.9	Dead		f.tr.	680	
		C	3.1	1.8	2.4	2.6	2.2	3.2	"		2.6	500	
		T	3.1	1.8	2.4	2.6	2.2	4.1	"		2.7	1180	
518	24	F	f.tr.	v.f.tr.	f.tr.	Dead					f.tr.		
		C	3.7	1.5	3.9	"					3.0		
		T	3.7	1.5	3.9	"					3.0		
533	38	F	v.f.tr.	1.2	f.tr.	"					tr.		
		C	4.4	4.7	3.9	"					4.3		
		T	4.4	5.9	3.9	"					4.7		
523	32	F	0.5	v.f.tr.	f.tr.	f.tr.	tr.	Dead			f.tr.		
		C	4.8	2.2	4.5	4.9	5.7	"			4.4		
		T	5.3	2.2	4.5	4.9	5.7	"			4.5		
532	49	F	v.f.tr.	v.f.tr.	f.tr.	f.tr.	v.f.tr.	"			f.tr.		
		C	4.1	1.4	6.1	7.1	3.6	"			4.5		
		T	4.1	1.4	6.1	7.1	3.6	"			4.5		
519	35	F		0.6	tr.	tr.	tr.	"			tr.	357	
		C		4.0	6.0	6.6	2.3	"			4.7	443	
		T		4.6	6.0	6.6	2.3	"			6.5	800	
522	27	F	f.tr.	2.9	0.9	4.3	Dead				2.7		
		C	3.6	6.9	6.7	8.7	"				6.5		
		T	3.6	9.8	7.6	13.0	"				9.2		
530	72	F	1.4	2.0	2.2	1.2	1.4	1.2	1.7	0.7	1.5	813	770
		C	2.5	2.2	5.0	3.2	3.1	1.7	1.8	1.1	2.6	22	280
		T	3.9	4.2	7.2	4.4	4.5	2.9	2.5	1.8	4.1	835	1050

F.tr., faint trace; v.f.tr., very faint trace. F, free sulfapyridine. C, conjugated sulfapyridine. T, total of free and conjugated sulfapyridine.

Date of infection: 10/15/38.

From 10/20/38 to 12/6/38 blood was obtained 8 hours after feeding 600 mg. of sulfapyridine. On 12/17/38 blood was obtained 9 hours after feeding 600 mg. of sulfapyridine.

Urines were obtained on various dates.

When the tissues of these animals dying at various intervals were compared with those of the untreated control animals coming to

autopsy at the same time, no differences whatsoever could be found between the tuberculous involvement of the organs of the two groups. Numerous tubercles studded the cortices of the kidneys and there were large areas of caseous pneumonia in the lungs, such as were found in the untreated rabbits.

In Table V are shown the concentrations of free and combined sulfapyridine in the bloods and urines of animals receiving this drug. The contrast between these values and those found for sulfanilamide is striking. With such low blood levels in this group of animals, it is not at all surprising that the drug failed to exert any beneficial effect in the tuberculous animals. Unfortunately only a few urine determinations were made but on the whole, the levels in the urine were proportionately low.

Summary. Sulfapyridine administered in daily divided doses amounting to 750 mg. per kilo. of body weight had no inhibitory effect on the development of experimental tuberculosis in the rabbit. However, the concentration of sulfapyridine attained in the blood was much lower than that of sulfanilamide when the latter drug was administered in the same dosage.

Since the completion of this study a report has appeared by Feldman and Hinshaw (10) concerning the effect of sulfapyridine on the course of experimental tuberculosis in the guinea pig. Beginning treatment 3 days before infection with a human strain of bacilli and continuing treatment for 56 days afterward, these investigators found this drug exerted "a definite and striking modification and retardation in the expected course of the tuberculosis in the guinea pig." Since the weight of their animals was not mentioned, it is difficult to compare their dose with the one we administered to rabbits. However, had their animals weighed 400 gm., the amount administered would have been 1250 mg. per kilo. of body weight for 10 days, and 625 mg. per kilo. for the remaining 49 days. No mention was made of the concentration of the drug obtained in the blood.

THE VIABILITY OF TUBERCLE BACILLI FROM GUINEA PIGS TREATED WITH SULFANILAMIDE

In our previous report (1) we mentioned that tissue emulsions from guinea pigs that had been infected with virulent tubercle bacilli and

had then been treated with 500 mg. of sulfanilamide each day, had been injected into normal guinea pigs to test the viability and virulence of the tubercle bacilli. Under aseptic precautions one-half of each spleen and one whole bronchial lymph node from each of four treated animals were ground separately in a mortar through a copper wire screen. Several cubic centimeters of saline were added and 1 cc. of the resulting emulsion was injected into the groin of a normal adult guinea pig. These animals lived from 29 to 66 days.

When the lungs, livers, and spleens of these 8 animals were studied, both grossly and microscopically, tuberculous involvement was found in all of them. From this we can conclude that in animals that have been treated with large doses of sulfanilamide, over a period of 6 weeks after infection with the tubercle bacillus, although the progress of the infection is decidedly inhibited, viable bacilli capable of producing progressive tuberculosis are still present in the tissues.

THE EFFECT OF SULFANILAMIDE ON GUINEA PIGS INFECTED WITH TUBERCLE BACILLI THREE WEEKS BEFORE TREATMENT WAS BEGUN

In this experiment 28 adult male guinea pigs, averaging about 450 grams in weight, were infected in the groin with 0.1 cc. of a suspension of virulent human type tubercle bacilli. This suspension contained approximately 5–6 organisms per oil emersion field. Twenty-one days after infection treatment with sulfanilamide was begun in 14 of the animals. Each treated animal received in the cheek pouch 100 mg. at 9 a.m., 100 mg. at 2 p.m., 100 mg. at 7 p.m., and 200 mg. at midnight, a total of 500 mg. or 1100 mg. per kilo. of body weight. This treatment was continued for 23 days, at which time all the survivors were killed. The first treated animal died after 11 days of treatment, and 6 others died apparently from the toxic effects of the drug.

At autopsy there was rather extensive tuberculosis, and, no gross or microscopic difference could be noted between the extent of the lesions of the two groups. In this study sulfanilamide administered for 3 weeks to guinea pigs in which tuberculosis, initiated by a relatively large dose of virulent bacilli, had been progressing for 3 weeks, before treatment was begun exerted no detectable effect on the course of the disease. Whether a more slowly progressing infection initiated

by a smaller number of bacilli can be inhibited by more prolonged treatment, is a matter for further study. Ballon and Guernon (6) using a smaller infecting dose than ours, found that when approximately 1000 mg. of sulfanilamide per kilo. of body weight were administered to guinea pigs 5 and 10 days after infection, the drug did have an inhibitory effect. The experiment was terminated in five weeks. Greey and his coworkers (4) found no effect on experimental tuberculosis in guinea pigs, when 300 mg. of sulfanilamide (860 mg. per kilo.) were given each day, beginning 17 and 24 days after infection. The duration of the experiment is not stated.

DISCUSSION

The present study on the effect of sulfanilamide upon experimental tuberculosis in rabbits confirms the results of our previous study (1) on guinea pigs, demonstrating that adequate dosage of the drug, properly administered throughout the course of the infection, has a decided inhibitory effect upon the development of the disease. It must be remembered in evaluating the lesions in the present experiments that we chose the severe test of intravenous inoculation which delivers bacilli at once to all parts of the body, and initiates the development of innumerable lesions throughout the tissues from the very beginning of the experiment.

It is important to repeat that three precautions must be observed in experiments of this type if positive results are to be obtained. First, the total daily dosage must be adequate. Since both the guinea pig and the rabbit acetylate a much larger percentage of the drug than does the human being, and since, whether because of less absorption or greater excretion, a given dose fails to produce as high a concentration in the blood in these animals as in man, much larger doses must be given to them than to the human being in order to attain and to maintain a given blood concentration. Second, in order to keep an effective amount of the drug in the blood throughout each 24 hours, the total daily dose must be administered in divided doses. Finally, the drug must be given throughout the experiment. These conditions were outlined in our previous paper (1), and the subsequent investigators who have observed these precautions (3, 4, 6, 7) have confirmed the inhibitory effect that we described, while those who have neglected them have had negative results (2, 5).

The present experiments show plainly that even when an adequate daily amount of the drug is administered in divided doses, there will be marked differences in the concentration of the drug in the blood of different animals. We have made no attempt to determine whether this is due to differences in absorption, in excretion, or in both. Furthermore, different animals differ markedly in the degree to which they acetylate the drug, and this leads to marked differences in the amount of free sulfanilamide in their blood and tissues. It is of particular interest that on a given daily dosage, individual animals tend to maintain a high or a low concentration of the drug in the blood. There can be little doubt that these individual differences in the ability to maintain a high level of free sulfanilamide in the blood on a given daily dosage play an important rôle in determining the differences in the effectiveness of the treatment in different individual animals.

While sulfanilamide has in no case cured experimental tuberculosis, the demonstration of a marked inhibitory effect upon the proliferation of the bacilli in these as well as in our previous experiments, and in the experiments of others who have observed the proper conditions of dosage, is a matter of considerable interest for several reasons. In the first place, the animal species chosen for these experiments (guinea pigs, rabbits) have an extreme degree of susceptibility to the tubercle bacilli used in the respective experiments; large numbers of highly virulent bacilli were injected; and in the present studies the route of infection was the most favorable one for the bacteria. Under these conditions, even hemolytic streptococci, which sulfanilamide is so effective in controlling in the more resistant human being, have proliferated for a time and have remained alive in the highly susceptible mouse during treatment with the drug; and, though the growth of the bacteria was inhibited during the period of treatment, when the administration of the drug was stopped the bacteria began to proliferate again and killed the animals (11). In the case of the effect of sulfapyridine on various strains of pneumococci a similar situation holds (12). Indeed, the evidence is very strong that in the human being the therapeutic value of this drug resides only in an inhibitory effect upon the proliferation of the bacteria, and that sterilization of the tissues depends upon acquired resistance developed by the patient while the proliferation of the bacteria is held in check (13). Sulfapyridine is ineffective in curing the highly susceptible mouse from

infection with certain types of pneumococci, whereas in the more resistant human being the inhibitory effect of the drug serves to bring about recovery.

Even though sulfanilamide may prove to be of no appreciable therapeutic value in the treatment of human tuberculosis, the fact that it has a definite inhibitory effect upon the tubercle bacilli in the highly susceptible guinea pig and rabbit under conditions most favorable for the bacillus, suggests the possibility that a modification of the structure of the compound may enhance its activity against the tubercle bacillus in a manner comparable to the effectiveness of sulfapyridine in the treatment of infection with types of pneumococci on which sulfanilamide exerts little effect.

In the rabbits infected with bovine tubercle bacilli and treated with sulfapyridine, no inhibitory effect on the progress of the infection was evident. Feldman and Hinshaw (10), however, have reported a definite inhibitory effect of this drug on infection with human type bacilli in the guinea pig. Our negative experiments do not conflict with their positive results, for our conditions were different. Regardless of any other considerations, the concentration of the drug in the blood of our rabbits was so low that it could hardly have been expected to have exerted any influence upon the infection. The rabbit is clearly an unsuitable animal for the study of the action of sulfapyridine administered by mouth, and we would draw no general conclusions as to the effect of this drug upon tuberculous infection from our experience with its action in rabbits.

SUMMARY

The present study of the effect of sulfanilamide on experimental tuberculosis in the rabbit confirms the result of our previous study on the guinea pig, demonstrating that adequate dosage of the drug, properly administered, has a definite inhibitory effect upon the development of the disease.

Individual rabbits exhibit marked differences in the degree to which they acetylate the drug, and in their ability to attain and maintain a high concentration of the drug in the blood following the administration of a given dosage. These differences undoubtedly play a rôle in determining the degree to which a given dosage of the drug will

exert a beneficial effect upon the progress of the infection in different animals.

We wish to stress the fact that no conclusions regarding the effect of the drug upon human tuberculosis should be drawn from these experiments. That is a problem that requires a separate and carefully controlled study.

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EXPLANATION OF ILLUSTRATIONS

(Photographs by Mr. Milton Kougl)

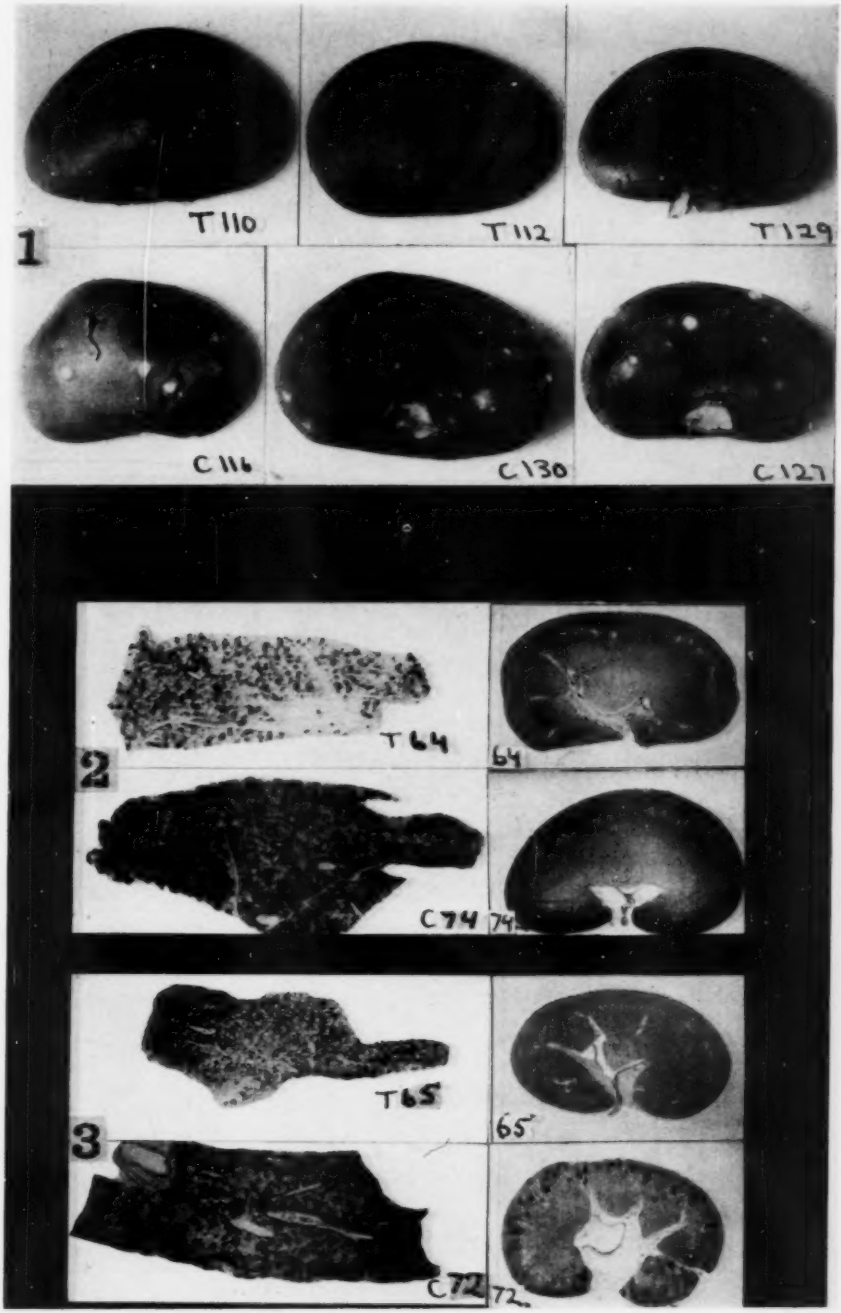
PLATE I

FIG. 1. Kidneys from rabbits of Series II treated with 600 mg. per kilo. of sulfanilamide daily (T) and their untreated controls (C). Nos. 110 (T) and 116 (C) autopsied 64 days after infection. Nos. 112 (T) and 130 (C) autopsied 72 days after infection. Nos. 129 (T) and 127 (C) autopsied 79 days after infection. The dark dents on the surface are scars which are so commonly found in the kidneys of stock adult rabbits. The white areas are tuberculosis.

FIGS. 2 AND 3. Lungs and kidneys from rabbits of Series III treated with 750 mg. per kilo. of sulfanilamide daily (T) and their untreated controls (C).

FIG. 2. Nos. 64 (T) and 74 (C) autopsied 21 days after infection.

FIG. 3. Nos. 65 (T) and 72 (C) autopsied 24 days after infection.


1
T110
T112
T129
C116
C130
C127
2
T64
64
C74
74
3
T65
65
C72
72

PLATES II AND III

Lungs and kidneys of rabbits treated with 750 mg. per kilo. of sulfanilamide daily (T) and their untreated controls (C). The lungs of all animals except Nos. 503 and 509 were inflated. The tubercles in these latter animals appear, therefore, more closely set than those in which inflation had been carried out. The average of weekly determinations of the concentration of free sulfanilamide in the blood is indicated after the letter F on the respective photographs.

FIG. 4. Nos. 503 (T) and 526 (C) autopsied 33 days after infection.

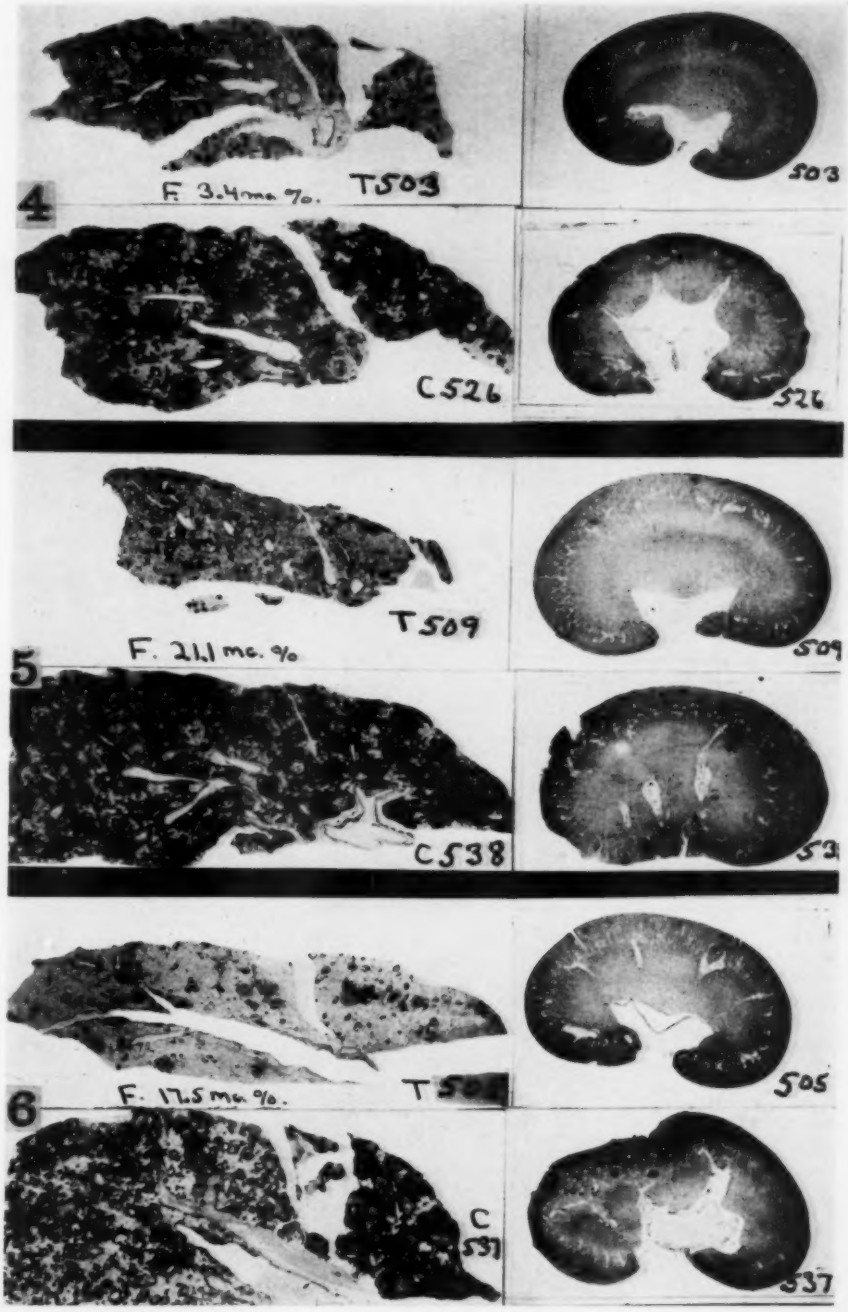
FIG. 5. Nos. 509 (T) and 538 (C) autopsied 35 days after infection.

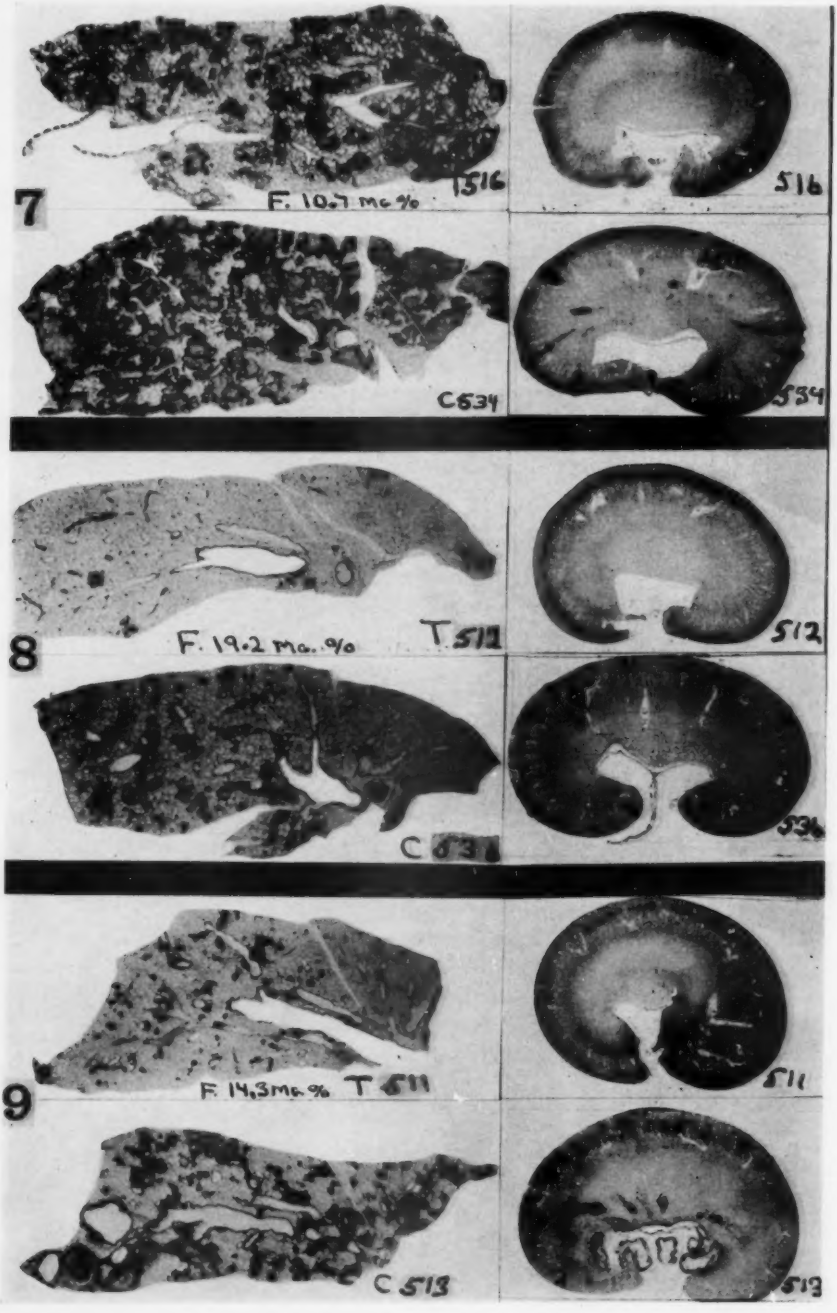
FIG. 6. Nos. 505 (T) and 537 (C) autopsied 38 days after infection.

FIG. 7. Nos. 516 (T) and 534 (C) autopsied 53 days after infection.

FIG. 8. Nos. 512 (T) and 536 (C) autopsied 55 days after infection.

FIG. 9. Nos. 511 (T) and 513 (C) autopsied 72 days after infection.


4
F. 3.4 mg. %.
T503
503
C526
526
T509
F. 21.1 mg. %
509
5
C538
538
F. 17.5 mg. %.
505
6
C
537
537


7
F. 10.7 mg %
1516
516
C 534
534
8
F. 19.2 mg. %
T 512
512
C 536
536
9
F. 14.3 mg. %
T 511
511
C 513
513

NERVE ENDINGS IN THE CARDIAC MUSCLE OF THE RAT

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By using a modification of the method of vital staining with methylene blue, nerve endings were studied in the cardiac muscle of the rat. It was possible to oxidize the dye adequately in frozen sections, and a comprehensive view of the arrangement of the nerves could be obtained in a few slides.

REVIEW OF LITERATURE

Huber and de Witt in 1897 reviewed the subject of cardiac innervation, and using methylene blue as a vital stain, they demonstrated an intraauricular plexus of nerve fibers. Nerves traced to the auricles were found to end as bulbs, either in the sarcolemma or closely applied to the muscle nuclei. No endings were described in the ventricles nor were muscle spindles seen anywhere.

Smirnow (1900) likewise employed methylene blue and described nerve filaments winding between muscle fibers and ending on the surface or within the sarcoplasm.

Wilson (1909) observed nerve fibers travelling down the auriculoventricular bundle in mammals. Endings were seen around the Purkinje cells and in the ventricular muscle, but no spindles were found. Sensory endings were identified in the fibrous coat of arteries. These endings were of the arborizing type with no capsule.

Meiklejohn (1913), using a silver technique, demonstrated clearly the innervation of the nodal tissue of mammalian hearts. The cardiac muscle is richly innervated in this area and complicated endings were found around the nodal nuclei and Purkinje fibers.

Woollard (1926), employing methylene blue, found ganglia in all parts of the heart, although they were most common in the auricles

[1]Henry Strong Denison Scholar for 1938–39.

and around the auriculo-ventricular node. The cells were all subendocardial and were rare in the ventricle. The motor nerves formed a fine plexus around the muscle fibers. Terminal branches pierced the sarcolemma where they ended in knobs and loops. This finding was confirmed in cross sections of the muscle. A few endings were seen in the ventricles. Sensory endings of a bush-like configuration were found in the subpericardial and subendocardial tissues. Nerve endings on fat cells were also described by Woollard.

Lawrentjew (1927) found that cutting the vagus nerves caused the sensory endings in the endocardium to degenerate. Okamura (1929) described three types of nerve cells in the heart.

Boeke (1925, 1933), by means of a modified Bielschowsky technique, studied the nerve endings on the bundle of His in the tortoise heart. He found a great number of sensory endings in the endocardium. Motor endings were seen close to the nuclei of the muscle fibers in all parts of the heart.

Greene and Maneval (1933) approached the subject of sympathetic nerve endings in the heart from the pharmacological standpoint. After observing the response of isolated strips of turtle heart in adrenalin solution they concluded that the greatest number of endings were around the sinus node. The auricles responded slightly, but adrenalin elicited little or no response from the ventricles.

Nettleship (1936) described large, diffuse sensory nerve endings in the subendocardium of both auricles and ventricles. Section of the vagus nerves caused degeneration of these endings.

Schimert (1938) was unable to differentiate sympathetic and parasympathetic fibers on a morphological basis. He found that the left sympathetic chain supplied the posterior and the right anterior surface of the heart. Sensory fibers ran in the vagus nerves to the stellate ganglia.

Plechkova (1936) is said to have described sensory spindles in the heart muscle. The paper was published in Russia and we have been able to obtain only a brief abstract.

METHOD

Methylene blue (*nach Ehrlich*) was dissolved in physiological saline to make a one per cent solution. It was freshly prepared for each injection and was heated to 100°F. Fifty cc. was the maximal amount required for a single animal. The

rat was anesthetized with ether and the abdomen opened widely. The dye was slowly injected, at a rate of one or two cc. per minute, from a syringe whose needle was inserted into the inferior vena cava.

The visible viscera and mucous membranes turned blue almost immediately after the injection was begun. If respiration became slow and labored, the injection was slowed until the animal breathed regularly again. Too rapid introduction of the dye was found to cause complete respiratory failure. Usually between 10 and 20 cc. of the solution could be injected before death. The perfusion was continued as long as the heart was seen to beat.

After death the thorax was opened, and the heart was removed and frozen *in toto* upon the microtome. Sections about 50 μ thick were cut from any desired area. The temperature of the block was controlled so that the sections did not tend to curl.

The surface of the frozen block of heart had its normal red color. After a section was cut and thawed, it turned an intense blue. Usually only ten seconds was required for this oxidation. The sections were then placed in 10 per cent ammonium molybdate solution and allowed to remain over night in the ice box.

The sections were cleared in glycerine, half an hour being sufficient for this process. They were then placed in two changes of 95 per cent, and one of 100 per cent alcohol. Fifteen minutes was found to be sufficient for the passage through the alcohols. They were cleared in xylol and mounted in balsam. During the dehydration the sections became folded and brittle and had to be straightened later. A liberal amount of balsam was used and the cover slip flattened with a screw clamp which was left in position over night. The next morning the clamp was removed and the cover slip replaced.

The nerve tissues remain well stained for about four months.

EXPERIMENTAL OBSERVATIONS

1. Nerve cells in the heart

Ganglia have been found in both the auricles and ventricles. In the ventricles, most of the nerve cells are near the auriculo-ventricular junction but small groups or individual cells have been seen close to the apex of the left ventricle.

Ganglia occur both subendocardially, and deep in the muscle walls. The majority of the intramuscular ganglia are situated in the left ventricle, but a few examples have been observed in cross sections of the auricles. Ganglia just beneath the endocardium are common. The cells often show a long axon arising from an axon hillock. A sketch (fig. 12) of the large motor cells demonstrates the granular nature of the cytoplasm, the large central nucleus which stains lightly, and the axon hillock.

There are other cells, about half the size of those just described, which have a pear-like shape with one end pulled out to a point. Their cytoplasm is finely granular, with a small nucleus. These cells form groups deep in the heart muscle and are not associated with the large multipolar cells.

2. Nerve fibers

Myelinated and non-myelinated nerve fibers can be recognized in the preparations. The myelinated fibers are found terminating in complicated endings which are thought to be sensory. Myelin itself does not stain, but appears as clear, refractile material around the axons.

The non-myelinated fibers are connected with the simpler motor endings and are seen as thin blue lines running between the muscle bundles. These non-medullated axons are shown in the pictures of motor endings (fig. 11).

No large nerve trunks are present in the heart muscle. The plexus of fibers follows the blood vessels almost to the point where the nerve endings are situated. They then branch off singly and plunge for a short course into the muscle.

3. Motor endings

Nerve endings of simple structure which probably have a motor function, are present in both the auricles and ventricles but are found most commonly in the ventricles. These endings always arise from non-medullated axons. Several varieties of ending have been noted, the end organ being either on the sarcolemma or within the sarcoplasm. They may be grouped in the following manner.

A. In the first type the axon ends in motor plates which lie on the surface of the muscle cells (figs. 11, 2 and 7). However our sections do not show enough detail to determine whether they are epilemmal or hypolemmal. A single axon often splits into filaments which supply two or three muscle fibers. The illustration shows branches of a single fiber ending on two muscle cells (fig. 11).

These endings are less complicated but in many ways are similar to those described as motor plates in striated muscle. They are common and a good preparation will show many in each section.

B. Endings inside the sarcoplasm are also numerous. They are best illustrated by drawings, and typical examples are shown in figures 2, 5 and 6. The axon branches into several filaments which end as a series of little bulbs, either free in the protoplasm or in close contact with the nuclei. This observation was checked by observing cross sections of the muscle fibers in which the intraprotoplasmic nature of the ending was established.

C. "En grappe" motor endings, somewhat similar to those described in skeletal muscle, were observed a few times. They are apparently quite rare and were seen only in the ventricle. A sketch (fig. 4), shows an ending with three prong-like projections extending into the protoplasm.

D. Nerve fibers have been seen to end on Rouget cells in capillary walls. Only one cell out of many in the vessel wall has such an innervation. The axon terminates in a small bulb in close approximation to the nucleus of the cell (fig. 1).

Nerves supplying cardiac muscle follow the blood vessels closely until the fibers are almost at their termination as shown in figure 3. The nerves are seen ending on muscle fibers which lie close to the blood vessel.

The motor endings were most numerous in the region just below the auriculo-ventricular junction. In sections closer to the apex, they were much less frequent. None was seen close to the apex of the ventricles.

Many of the muscle fibers apparently received an innervation from two different axons. One axon would end on the surface of the fiber, while a short distance away, another separate nerve would terminate within the sarcoplasm, often about the nucleus. A single fiber was never seen to split into two strands, one of which ended on the surface, while the other entered the sarcolemma. Two separate fibers were always present (figs. 2 and 6).

4. Complicated nerve endings of sensory type

Several varieties of complicated endings were seen which were interpreted as being sensory in nature. They originated from myelinated fibers, and were large enough to be seen easily.

A. Small encapsulated endings. The nerve fiber ended in a cy-

lindrical whorl, which was completely surrounded by a thin transparent capsule. This type was found quite frequently in the ventricles, lying between the muscle bundles. A common location was between layers of muscle, the fibers of which were running in different directions. The ending appeared wedged in the intervening space left by the overlapping of the layers. A sketch (fig. 9) of the ending demonstrates some of the detail. The medullated fiber, the clear capsule, the maze of interwinding fibers and the muscle bundles running in divergent directions are pictured.

Several varieties of encapsulated endings were seen which differ a little in size and shape. These have a very similar structure to that just described, but they are located between parallel muscle fibers.

B. Muscle spindles were demonstrated in the ventricles; these also showed considerable variation in their complexity. Large endings, situated within a thin capsule, whose termination is composed of a complicated mass of nerve fibers, and small strands of muscle, were occasionally found. Only a few endings of this type have been observed in the ventricle, and none in the auricle. They are of large size and occupy the entire field of a high power lens.

Somewhat simpler endings were seen where the nerve fiber broke

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FIG. 1. ENDING ON ROUGET CELL. × 1350

FIG. 2. DOUBLE INNERVATION OF MUSCLE

One ending on surface, other near the nucleus. × 1350.

FIG. 3. NERVE ENDINGS ON MUSCLES IN CLOSE APPROXIMATION TO BLOOD VESSEL. × 1350

FIG. 4. EN GRAPPE ENDING. × 1350

FIG. 5. MOTOR ENDINGS NEAR NUCLEUS AND IN SARCOPLASM. × 1350

FIG. 6. CROSS SECTION OF MUSCLE, SHOWING DOUBLE SET OF MOTOR ENDINGS. × 1350

FIG. 7. SINGLE NERVE ENDING ON TWO MUSCLE FIBERS. × 1350

FIG. 8. ENDING ON FAT CELL. × 1350

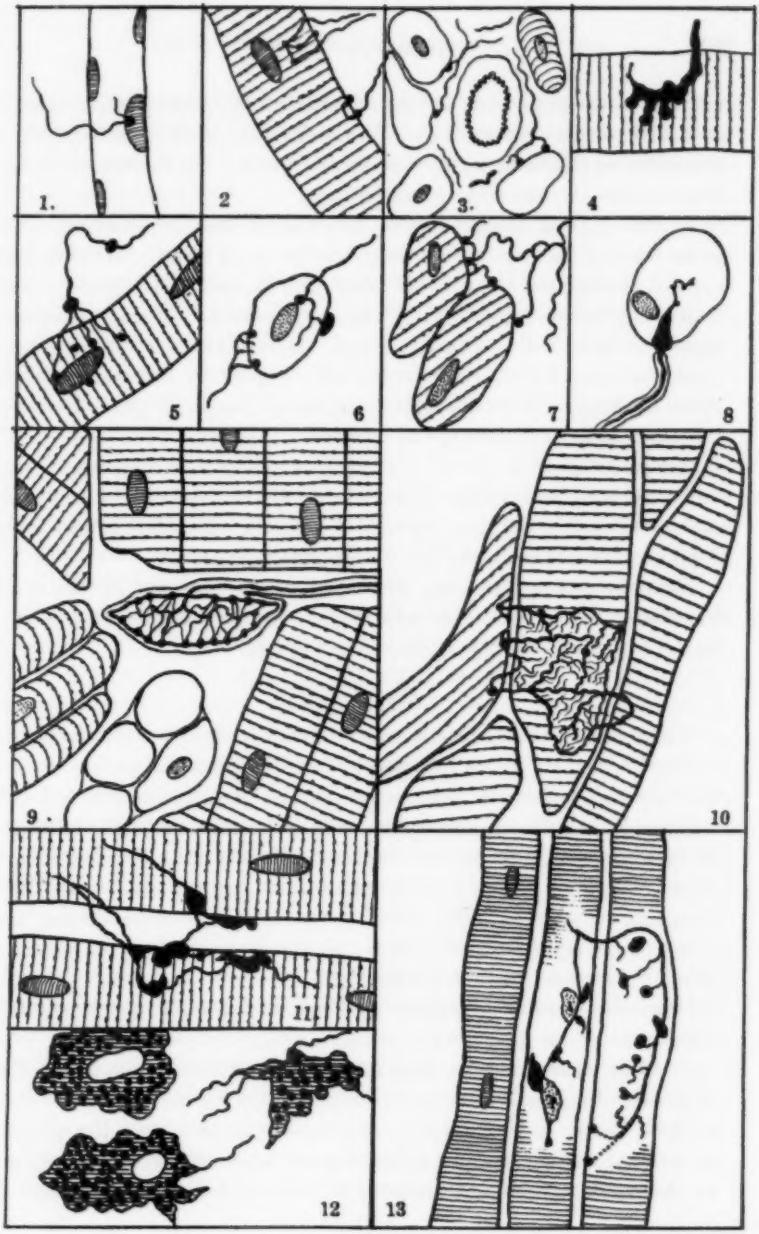
FIG. 9. ENCAPSULATED ENDING, LYING BETWEEN LAYERS OF MUSCLES RUNNING IN VARIOUS DIRECTIONS. × 1350

FIG. 10. NON-ENCAPSULATED MUSCLE SPINDLE. × 1350

FIG. 11. COMPLICATED MOTOR ENDING, TWO MUSCLES INVOLVED. × 1350

FIG. 12. DETAIL OF CYTOPLASM OF LARGE MOTOR CELLS. × 1350

FIG. 13. LARGE SENSORY ENDING. × 315



FIGS. 1 to 13

up into a number of fine strands which wrapped themselves closely around a single muscle fiber. The individual fibers extended for a considerable distance, winding back and forth. No definite terminations of the strands were recognized.

A third type is similar to the last, except that the nerve strands wrap around the muscle fiber only two or three times. Neither this type of ending, nor the one described previously has a capsule. The axons supplying these endings are myelinated. The terminations appear to be of the nature of a muscle spindle, but of a much simpler type than the first variety described. Figure 10 illustrates one of these endings. They are quite commonly found in the ventricles, but none have been seen in the auricles.

Figure 13 is a sketch of a rather complicated ending which terminates upon two muscle fibers. It is not the usual type of motor end plate and is perhaps sensory in nature. Similar endings have been seen rather infrequently in the ventricle.

Terminations on fat cells, similar to those described by Woollard have been observed. The axon which supplies the ending is myelinated. A sketch (fig. 8) of one example is shown.

DISCUSSION

Many muscle fibers in the heart are innervated by two separate endings. The ending of one nerve fiber lies upon the surface of the sarcoplasm whereas the other fiber pierces the sarcolemma and terminates in a bulb which is often in close proximity to the nucleus. In view of the innervation of the heart by both the sympathetic and parasympathetic components of the autonomic nervous system it is tempting to consider that one of these fibers is of sympathetic, the other of parasympathetic origin. At the present time we have no proof to support this hypothesis.

With the possible exception of Plechkova no one has previously demonstrated in the cardiac muscle, sensory endings of the type resembling neuromuscular spindles. In the present preparations this type of ending was found quite frequently in the muscle of the ventricles. These endings which are less complicated than the spindles of striated muscle, appear to be of a structure which could respond to the stretch of muscle contraction. The gradual accumulation of

data makes it more and more evident that stretch receptors are present in the smooth muscle of the thoracic and abdominal viscera. It is probable that some types of spindles will eventually be demonstrated in all types of muscle.

The spindles found in cardiac muscle are, in regard to their complexity, of a transitional type between those observed in striated and smooth muscle. The stretch receptors in smooth muscle have no capsule, while those in striated muscle are of great complexity and are usually surrounded by a heavy covering of connective tissue, although this is not always the case. Certain of the spindles seen in cardiac muscle have a thin capsule and others of simpler type have no demonstrable sheath.

No free nerve endings of the type described in the skin, nor of the arborizing variety described by Nettleship in the endocardium, have been observed in the myocardium. Nerve fibers have been seen which appeared to end in bulb-like projections, lying between the muscle bundles. They occurred singly or in small groups. It was impossible to be certain that they were not axons which were observed when cut obliquely or in cross section.

There has always been great interest in the manner in which anginal pain arises in the heart. Many feel that the pain is due to ischemia of the cardiac muscle. Lewis states that the products of abnormal muscular metabolism diffuse out and stimulate the nerve endings in the intermuscular spaces. The nerve endings and nerves which carry the pain have not been demonstrated.

Recently Singer (1927, 1935), and Katz and his coworkers (1935) have advanced a theory that nerve endings in the walls of the blood vessels are stimulated, causing the pain. Their experimental studies involved a technique of extreme delicacy, removing the adventitia from a segment of coronary artery while leaving the arterial nerve trunks intact. From our studies this operation appears very difficult and there is little objective evidence to support their hypothesis. There are undoubtedly sensory endings in the walls of arteries.

The encapsulated endings and complicated endings upon the muscle fibers that we have demonstrated, are interpreted by analogy as sensory receptors probably responding to stretch stimuli. There is no evidence that they normally respond to pain stimuli.

However it is possible that all sensory receptors react to stimuli of abnormal strength in a manner which is interpreted as pain. If it is assumed that the complicated endings in the myocardium are concerned with the normal functioning of the heart, it is not inconceivable that they may be severely stimulated during violent effort or during ischemic states. This may cause an overflow of stimuli in the posterior horns of the spinal cord, stimulating the cells mediating pain in the skin and deep tissues and producing the typical cardiac pain. Thus the pain over the chest wall and down the arms might be experienced in the absence of specific pain fibers coming from the heart itself.

The method of staining employed in these observations, for the first time makes use of frozen sections in this particular vital technique. The use of the freezing microtome to bring about the oxidation of methylene blue is rapid, sure, and easily carried out. Sections can be made of muscular tissue which are thin enough to be fully oxidized, yet thick enough to give a three dimensional view of the nerve endings. It would seem practical to use this method to study the innervation of any type of tissue too thick *in toto* to permit perfect oxidation.

SUMMARY

1. By making frozen sections of tissue perfused during life with methylene blue, it is possible to oxidize the dye adequately and demonstrate nerve cells, nerve fibers and nerve endings.

2. Several types of nerve endings were found in the myocardium of the rat; some were interpreted as motor, others as sensory. Complicated terminations encircling muscle fibers, were considered to be stretch receptors. These have not been demonstrated before in cardiac muscle.

3. The mechanism of cardiac pain is discussed. It is suggested that abnormal stimulation of the complicated sensory endings might influence the pain neurones in the posterior gray columns of the cord and produce abnormal sensations interpreted as pain in the chest and arms.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of members of the Editorial Board of this Journal.)

Rural Medicine: Proceedings of the conference held at Cooperstown, New York, October 7 and 8, 1938. 268 pp. Diagrams. $3.50. *Charles C. Thomas, Springfield, Illinois, 1939.*

This book presents the papers read and discussed at a conference on rural medicine organized by the staff of the Mary Imogene Bassett Hospital. The purposes of the conference were the following: To provide a forum for discussion of problems of rural medicine and to give publicity to ideas and suggestions for accelerating progress in rural hygiene. To define and set apart as distinct problems those which have a special relationship to the health of rural communities. To present a true picture of rural morbidity, as far as the experience of a single rural hospital may serve for this purpose. To stimulate unsentimental scientific scrutiny of those conditions which influence the incidence of disease and disability in rural populations and to apply such scrutiny to the problems of rural medicine.

The conference brought together a noteworthy gathering of experts on public health, medical education, organization of medical service and rural life. It held four sessions, devoted respectively to rural morbidity, health department programs and school health programs in rural areas, post graduate medical education in rural areas and economics of rural medicine. At these sessions papers were presented by members of the staff of the Bassett Hospital, by local physicians, by biostatiticians who had studied the local community and by experts on the various topics discussed. The volume contains sixteen papers and the excellent discussions which they evoked, and is concluded by an extensive bibliography of rural medicine.

This collection of papers raises many questions regarding rural medicine and answers conclusively but a few of them. It demonstrates clearly however that plans for the improvement of medical service must be adaptable to local needs and must have sufficient flexibility to meet many variations in local conditions and attitudes in order to be effective. The problems of rural medicine are set forth from a variety of aspects, and those interested in the changing conditions of medical care should study the excellent approach to the subject which this book represents, namely an effort to study at first hand and at close range one phase of the problem of medical service by a distinguished group of leaders in public health and medical practice.

The discussion of economics of rural medicine is noteworthy. Beginning with accounts of experience in this field, the discussion is continued by close observations and sensible interpretations of the local situation followed by a broad general discussion both from a liberal and from a more conservative point of view.

The conference reported in this book is an excellent example of the educational process which is essential, if intelligent cooperation is to be effected between the public and the medical profession, so much needed in working out the details of any program of medical service which may be advanced by legislation. Dr. George Mackenzie and his colleagues of the Mary Imogene Bassett Hospital deserve much credit for inaugurating such a valuable method of stimulating sound thinking based on facts, which is especially valuable at this time of controversy regarding medical service.

G. C. R.

The Vasomotor System in Anoxia and Asphyxia. By ERNST GELLHORN and EDWARD H. LAMBERT. 71 pp. Illus. $1.00. *University of Illinois Press, Urbana, 1939.* (*Illinois Medical & Dental Monographs, Vol. II, No. 3.*)

In this monograph of seventy-one pages Dr. Gellhorn and Dr. Lambert present the results of their study of the effects of oxygen deficiency and carbon dioxide excess on the vasomotor system. Recognizing that blood pressure determinations are difficult to interpret during anoxia due to the simultaneous changes in respiration, these authors devised experiments to eliminate that variable. From their experiments, they conclude that anoxia causes a rise in blood pressure by action on the carotid and aortic bodies, while carbon dioxide excess has its primary effect on the vasomotor center and only a minor one on the peripheral chemoreceptors.

The monograph is well written, illustrated with many graphic records of the authors' experiments, and reviews the current literature pertaining to the subject. The relationship of their findings to the need of the animal to maintain a constant internal environment is also discussed in an excellent manner.

C. L. G.

Physiology of Exercise. By JAMES HUFF MCCURDY and LEONARD A. LARSON. 3rd ed. revised. 349 pp. $3.75. *Lea and Febiger, Philadelphia, 1939.*

In addition to considerable revision of the text, the authors have added two new chapters in their third edition. One of the new chapters deals with the effects of exercise in people over forty years of age and the other with the problems of physical exercise in relation to women.

This book summarizes the findings of the many experiments which have been made during and after exercise. Numerous references are given at the end of each chapter. However, there has been no attempt to give adequate physiological explanations of these findings. As a text book for facts concerning exercise, this book is excellent; as a "physiology" of exercise, it leaves much to be desired.

C. L. G.

Surgical Treatment of Hand and Forearm Infections. By A. C. J. BRICKEL. 300 pp. Illus. $7.50. *C. V. Mosby Company, St. Louis, 1939.*

Excellent drawings, photographs, and roentgenograms make this book a valuable contribution to the study of surgical conditions of the hand. Kanavel's

original monograph and Hart's article in Lewis' System of Surgery embody the fundamentals of this problem. Brickel's only disagreement with these authors lies in his interpretation of the palmar spaces. Brickel could not demonstrate a separate mid-palmar space medial to the origin of the adductor pollicis. He dissected hands after injection with radiopaque material and found that the proximal part of the palmar cavity is a common space which contains and is usually completely filled by the ulnar bursa and its enclosed tendons; and that the distal part is divided into compartments by the septa separating the lumbrical canals and tendon sheaths.

Plate III illustrates this relationship by showing a cross section of the hand at the level of the metacarpophalangeal joint of the thumb giving a clear picture of the disposition of the palmar fascia and the interrelationships of all parts of the hand.

The proper locations of incisions to relieve infections and the control of hemorrhage are discussed, stressing the importance of adequate exposure without damage to important structures and with least interference with function.

I. R. T.

Osler's Principles and Practice of Medicine. 13th ed. Revised by HENRY A. CHRISTIAN. 1424 pp. $9.00. *D. Appleton-Century Company, New York, 1938.*

The first edition of Osler's Practice appeared in 1892; the present edition is the thirteenth. The first seven editions were edited by Osler; the next five by Osler and McCrae; the following four, appearing after the daeth of Osler, by McCrae; the present volume, the first to appear after the death of McCrae, by Christian. This is an astonishing and noteworthy record, unequalled, I believe, by that of any modern medical text. There must be solid worth in a book that for nearly fifty years holds an established place at the top of the list of treatises on the practice of medicine. This eminence was due, in the first place, to its excellence as it came from the hands of the most distinguished physician and teacher of his day; and the high position thus won has been maintained by the judicious and painstaking care with which his pupil, McCrae, has revised successive editions. And now to continue the tradition, Christian, a pupil of Osler and friend and colleague of McCrae, is selected to revise the thirteenth and subsequent editions.

It is no easy task to take a great book and in successive revisions to keep it abreast of rapidly changing knowledge and yet to preserve its spirit. And during these forty-six years what amazing change and advance has occurred! It is incredible that endless alterations and additions could be incorporated within a form whose beauty and charm depended largely upon its well rounded and highly ornamented completeness. The thirteenth Osler is not the same book as the first, it has lost some of its freshness and charm. However, this loss was inevitable and the wonder is that it has retained as much of it as it has. I do not pretend that I have read the thirteenth edition through but it has been upon my table for months and I have referred to it again and again, reading the chapters pertaining

to topics which at the moment interested me. The information is terse and reliable and as complete as anyone could expect it to be in what of necessity is a synopsis. It is a safe guide for the student and a satisfactory compendium for the practitioner wishing hastily to refresh his memory or to orient his knowledge of a field forgotten or not previously explored.

A problem that now confronts the student and practitioner, about to select a text book of medicine, is to decide between the rival claims of a text written by one author and a text written by many authors, presumably each a specialist in the subject to which he contributes. The former has the advantage of uniformity and proportion, the latter of precise and authoritative information, especially about matters still in dispute. The fact that both kinds of books are popular demonstrates that there is a difference of opinion on this point. However, preference is decided usually by the quality of the book rather than its form and at times the inspiration and enthusiasm of a great teacher may outweigh in value a more meticulously accurate compilation of information.

L. H.

BOOKS RECEIVED FOR REVIEW

Proctology for the General Practitioner. By FREDERICK C. SMITH. 386 pp. Illus. $4.50. *F. A. Davis Company, Philadelphia, 1939.*

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INDEX TO VOLUME LXV

Addison's disease, The coincidence of diabetes mellitus and. Effect of cortical extract on glycemia and glycosuria 456
Anniversary, The celebration of the fiftieth 40
Arbesman, Carl (see Eagle, Harry) 283

Baker, Benjamin M., Jr.: The relation of syphilis to nephritis 196
Ball, Eric G.: Chemical reactions of nicotinic acid amide *in vivo* 253
Bang, Frederik B. (see Gey, George O.) 393
Billings, John S.: The plans and purposes of the Johns Hopkins Hospital ... 7
Biskind, Gerson R. and Jerome Mark: The inactivation of testerosterone propionate and estrone in rats 212
Blackman, S. S., Jr.: Arteriosclerosis and partial obstruction of the main renal arteries in association with "essential" hypertension in man 353
Bloomfield, A. L.: The coincidence of diabetes mellitus and Addison's disease. Effect of cortical extract on glycemia and glycosuria 456
Book reviews 142, 218, 288, 349, 427, 500

Cardiac muscle of the rat, Nerve endings in the 489
Charity and knowledge 23
Culp, Ormond S.: Squamous metaplasia, simulating carcinoma, associated with prostatic infarction 239

Delfs, E. (see Shettles, Landrum B.) 419
Duff, G. Lyman: The clinical and pathological features of carcinoma of the body and tail of the pancreas 69

Eagle, Harry, Carl Arbesman and Walter L. Winkenwerder: The use of the low temperature ball mill for the preparation of pollen extracts 283
Epileptics, The electroencephalogram in healthy relatives of: The constitutional elements in "idiopathic epilepsy" 125
"Essential" hypertension in man, Arteriosclerosis and partial obstruction of the main renal arteries in association with 353

Follis, Richard H., Jr., and Arnold R. Rich: Further studies on the effect of sulfanilamide on experimental tuberculosis 466
Futcher, Palmer Howard, and Virgil C. Scott: Four cases of gonococcal endocarditis treated with sulfanilamide, with recovery of one 377

Gey, George O., and Frederik B. Bang: Experimental studies on the cultural behavior and the infectivity of lymphopathia venerea virus maintained in tissue culture 393
Gilman, Daniel C.: Charity and knowledge 23
Gonococcal endocarditis, Four cases of, treated with sulfanilamide with recovery of one 377
Graduate, To the 1
Grimmer, R. V. (see Langworthy, O. R.) 101

Harvey, A. M.: The action of procaine on neuro-muscular transmission 223
Hellman, L. M., and Landrum B. Shettles: Factors influencing plasma prothrombin in the newborn infant. I. Prematurity and vitamin K 138
Hellman, L. M. (see Shettles, Landrum B.) 419
Herrick, James Bryan: The Johns Hopkins Hospital; its past and its future. 56
Hospital, The opening of the 2
Howard, Evelyn: A note on the reactions of the mouse uterus to ovariectomy in the presence of the X zone 341
Hypotension, postural, Transient paralysis from 329

Iron metabolism in infancy. I. Factors influencing retention on ordinary diets 145
Iron metabolism in infancy. II. The retention and utilization of medicinal iron 167

Johns Hopkins Hospital, The plans and purposes of the 7
Johns Hopkins Hospital, The; its past and its future 56
Josephs, Hugh W.: Studies in vitamin A: Relation of vitamin A and carotene to serum lipids 112
Josephs, Hugh W.: Iron metabolism in infancy. I. Factors influencing retention on ordinary diets 145
Josephs, Hugh W.: Iron metabolism in infancy. II. The retention and utilization of medicinal iron 167

King, Arthur B.: Nerve endings in the cardiac muscle of the rat 489
King, Francis T.: Opening address 3

Langworthy, O. R., and R. V. Grimmer: A physiological study of the movements in palatal myoclonus 101
Lewis, Margaret R. (see Rich, Arnold R.) 291, 311
Loewenbach, Hans: The electroencephalogram in healthy relatives of epileptics: The constitutional elements in "idiopathic epilepsy" 125
Lymphocyte, The activity of the, in the body's reaction to foreign protein as established by the identification of the acute splenic tumor cell 311

Lymphopathia venerea virus, Experimental studies on the cultural behavior and the infectivity of, maintained in tissue culture 393

Mark, Jerome (see Biskind, Gerson R.) 212
Myeloblasts, The differentiation of, from lymphoblasts by their manner of locomotion 291
Myoclonus, palatal, A physiological study of the movements in 101

Nephritis, acute hemorrhagic, Electrocardiographic changes in 434
Nicotinic acid amide, Chemical reactions of, in vivo 253

Opening address 3

Pancreas, The clinical and pathological features of carcinoma of the body and tail of the 69
Pollen extracts, The use of the low temperature ball mill for the preparation of 283
Procaine, The action of, on neuro-muscular transmission 223
Prostatic infarction, Squamous metaplasia, simulating carcinoma, associated with 239
Prothrombin, plasma, Factors influencing, in the newborn infant. I. Prematurity and vitamin K 138
Prothrombin, plasma, Factors influencing, in the newborn infant. II. Antepartum and neonatal ingestion of vitamin K concentrate 419
Psychiatry, Relation of, to internal medicine 265

Rennie, Thomas A. C.: Relation of psychiatry to internal medicine 265
Rich, Arnold R., Maxwell M. Wintrobe and Margaret R. Lewis: The differentiation of myeloblasts from lymphoblasts by their manner of locomotion 291
Rich, Arnold R., Margaret R. Lewis and Maxwell M. Wintrobe: The Activity of the lymphocyte in the body's reaction to foreign protein as established by the identification of the acute splenic tumor cell 311
Rich, Arnold R. (see Follis, Richard H., Jr.) 466
Richardson, Arthur P.: Cyanosis in chickens and mice induced by sulfanilamide 445

Scott, Virgil C. (see Futcher, Palmer Howard) 377
Shettles, Landrum B. (see Hellman, L. M.) 138
Shettles, Landrum B., E. Delfs and L. M. Hellman: Factors influencing plasma prothrombin in the newborn infant. II. Antepartum and neonatal ingestion of vitamin K concentrate 419
Smith, Frank R.: Memorial notice 431

Sulfanilamide, Further studies on the effect of, on experimental tuberculosis. 466
Sulfanilamide, Cyanosis in chickens and mice induced by..... 445
Syphilis, The relation of, to nephritis..... 196

Testerosterone propionate and estrone, The inactivation of, in rats..... 212
Thomas, Henry M., Jr.: Transient paralysis from postural hypotension..... 329

Uterus, mouse, A note on the reactions of the, to ovariectomy in the presence of the X zone..... 341

Vitamin A, Studies in. Relation of vitamin A and carotene to serum lipids.. 112

Williams, Russell D.: Electrocardiographic changes in acute hemorrhagic nephritis..... 434
Winkenwerder, Walter L. (see Eagle, Harry)..... 283
Wintrobe, Maxwell M. (see Rich, Arnold R.).....291, 311

METHODS FOR QUANTITATIVE ESTIMATION OF THE VITAMINS

III. Measurement of Vitamin A Activity

● It was early recognized that vitamin A deprivation in animals resulted in cessation of growth or—if long continued—in the appearance of a characteristic eye condition known as xerophthalmia (1). These two pathologic effects were both utilized in the first methods proposed for quantitative estimation of this essential food factor.

The earliest techniques for determination of vitamin A were similar in that they all first provided for depletion of the body stores of vitamin A of the rat by restriction of the animals to basal rations free from or quite deficient in the vitamin. In the "rat growth" method, the vitamin A activity of the material under assay was estimated by feeding graded dosages to animals depleted of the vitamin (as gauged by cessation of growth) and recording the ensuing growth response (2). In the "curative technique," the incidence of xerophthalmia served as the criterion of vitamin A depletion (3), and vitamin A activity was estimated by determining the dosage of the test material necessary to establish cure of xerophthalmia.

Techniques were also gradually developed which in some instances embodied features of both the growth and curative methods. Still another technique based on the continuous appearance of cornified epithelial cells in vaginal smears—a further characteristic of vitamin A deficiency in female rats—was evolved (4). Further research showed that colorimetric and spectrographic methods may be adapted to the estimation of vitamin A activities of specific materials (5).

Of all methods for estimation of vitamin A in foods, the rat growth technique appears to be favored today (6). Gradual improvements and refinements—as well as recognition of the existence of provitamins A—have led to development of the growth method now included in the U. S. Pharmacopeia XI. This method requires that young rats weighing 40 to 50 grams (at an age not exceeding 28 days when placed on a vitamin A deficient ration) shall manifest symptoms characteristic of vitamin A deficiency within a period of 25 to 45 days. Rats properly depleted of vitamin A reserve are assembled in negative control groups receiving no supplement, reference groups receiving graded doses of the standard reference material, and assay groups receiving graded doses of the assay material. During the ensuing period of not less than 28 days, the test animals are fed daily doses of the proper supplements. The body weights of the animals are recorded at frequent intervals during and at the end of the assay period. From the average gains in body weight of rats in the assay and reference groups, dosages of assay and reference materials, and the vitamin A activity of the standard of reference, the vitamin A activity of the assay material is calculated.

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1938. Nutrition Abstracts and Reviews, 8, 281.

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